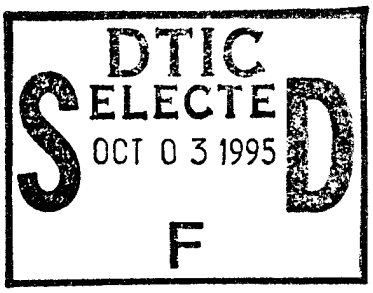
AD_____



CONTRACT NO: DAMD17-93-C-3061

TITLE: REVERSAL OF MULTIDRUG RESISTANCE IN BREAST CANCER

PRINCIPAL INVESTIGATOR: Lori J. Goldstein, M.D.

CONTRACTING ORGANIZATION: Fox Chase Cancer Center
American Oncologic Hospital
7701 Burholme Avenue
Philadelphia, Pennsylvania 19111

REPORT DATE: July 27, 1995

TYPE OF REPORT: Annual Report

PREPARED FOR:
U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 27, 1995	Annual (3/15/94 - 3/14/95)

4. TITLE AND SUBTITLE

Reversal of Multidrug Resistance in Breast Cancer

5. FUNDING NUMBERS

Contract Number

DAMD17-93-C-3061

6. AUTHOR(S)

Lori J. Goldstein, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia, PA 19111

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Our data show that MDR1 gene expression is important in breast cancer resistance. The role of the MDR1 gene in breast cancer treatment will be further defined by sequentially determining MDR1 gene expression pre and post treatment with doxorubicin in the context of three prospective clinical trials. In addition, this study will allow a correlation of MDR1 gene expression and clinical outcome. To determine what level of MDR1 gene expression is clinically significant, various molecular methods of determining MDR1 gene expression, including immunohistochemistry and quantitative reverse transcription followed by polymerase chain reaction, will be evaluated.

MDR can be reversed in vitro and we will test this hypothesis in a Phase I study of cyclosporin A and quinine as MDR reversers of vinblastine resistance. Together these studies will address the major goal of circumventing drug resistance in breast cancer. When the data of the MDR1 gene expression in breast cancer specimens from this proposal are available, clinical trials incorporating the modulators of MDR, cyclosporin and quinine, will be designed for breast cancer as well. An alteration in drug efflux potentially may have an impact on response to chemotherapy and may result in improved survival for breast cancer patients. During the period between March 15, 1993 and March 14, 1995, we have outfitted our laboratory with staff, equipment, supplies and reagents and have been performing control experiments and have been pursuing activation of the various clinical trials to support this project.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, Multidrug Resistance	71
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

INTRODUCTION

Drug resistance is a major obstacle in the treatment of cancer. The multidrug resistance gene (MDR1) encodes an energy dependent drug efflux pump, P170, that confers cellular resistance to multiple therapeutic agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins, taxol, and actinomycin-D. MDR1 gene expression is tumor specific in both *de novo* resistant tumors and those that acquire drug resistance following chemotherapy. The central role of P-170 in this multidrug resistance (MDR) phenotype suggests that modulation of either MDR1 gene expression or the function of P-170 may provide an effective means of clinically reversing drug resistance.

Our data show that MDR1 gene expression is important in breast cancer resistance. The role of the MDR1 gene in breast cancer treatment will be further defined by sequentially determining MDR1 gene expression pre- and post-treatment with doxorubicin in the context of prospective clinical trials. In addition, these studies will allow a correlation of MDR1 gene expression and clinical outcome. To determine what level of MDR1 gene expression is clinically significant, various molecular methods of determining MDR1 gene expression, including immunohistochemistry and quantitative reverse transcription followed by polymerase chain reaction, will be evaluated. We have also had the opportunity to analyze our samples for expression of the MRP gene (Multidrug Resistant Related Protein). The MRP gene is a new member of the ATP-binding cassette transporter superfamily. It is overexpressed in a variety of cell lines which exhibit acquired drug resistance, yet do not express P-glycoprotein. In addition, recent work has shown that this gene confers a multidrug resistant phenotype when transfected into sensitive cells. Currently, little is known of the mechanism by which MRP confers drug resistance. Furthermore, the clinical relevance of MRP is unknown.

MDR can be reversed *in vitro* and recent data from the *in vivo* transgenic mouse model suggests that combining MDR modulators such as cyclosporine and quinine, may have an advantage over either alone. We will test this hypothesis in a Phase I study of an analogue of cyclosporine A; PSC 833, and quinine as MDR reversers of vinblastine resistance. Together these studies will address the major goal of circumventing drug resistance in breast cancer. When the data of the MDR1 gene expression in breast cancer specimens from this proposal are available, clinical trials incorporating the modulators of MDR, cyclosporine and quinine, will be designed for breast cancer as well. An alteration in drug efflux potentially may have an impact on response to chemotherapy and may result in improved survival for breast cancer patients.

BODY

The aim of this project is to test the hypothesis that drug resistance in breast cancer is mediated by the MDR1 gene. Moreover, once MDR1 gene expression has been established in breast cancer and correlated with response and resistance to chemotherapy, such data may be used to predict drug resistance and design clinical trials to overcome such resistance using pharmacologic agents proven to reverse MDR *in vitro* and *in vivo.*

To accomplish the tasks outlined in the initial proposal, we have accomplished the following from March 15, 1993 to March 14, 1995:

1. Personnel

   a) Scientific Technician - Ken Geles, BS. Ken will be moving onto Graduate School at Northwestern University. He will be replaced by Rajashekar K. Reddy, MS.

   b) Post-Doctoral Associate - Dwayne Dexter, Ph.D.

   c) Research Fellow - Jack Leighton, M.D.,

2. Space and Facilities

   With the assistance of the personnel above, our 500 sq. ft. laboratory space has been fully equipped to perform the molecular experiments described in our original proposal.

3. Reagents and Supplies

With the assistance of the laboratory staff above, we have now fully equipped the laboratory with the necessary reagents and supplies. We have established reproducible, quality controlled experiments with regard to cell culture, RNA isolation, hybridization with our various cDNA probes and reverse transcription -PCR (RT-PCR) using our competitive template for the MDR gene as an internal control. In addition, the laboratory staff has been evaluated for proper handling of tissue specimens and RNA isolation by doing experiments of tumor bank specimens looking for MDR1 gene expression.

4. Methods

a) RNA Isolation. RNA is currently being isolated from tumor biopsies as previously reported. We initially were isolating RNA from fine-needle aspirates (FNAs) using a scaled down procedure, however, the yield of RNA recovered from these samples was inadequate for use in quantitative assays. Based on the size (or weight) of the sample, we were expecting to recover several μg of RNA, which would allow quantitative assays to be performed. To maximize RNA yield, we are employing a commercially available isolation kit (Qiagen, RNeasy Kit) which has greatly enhanced our recovery and yield from these small FNA specimens. This kit relies on a modified guanidinium salt lysis procedure combined with spin column chromatography technology to produce high quality RNA free of 5s RNA, DNA, and other contaminants. Using this procedure, we are typically recovering 3-8 μg of RNA from standard FNAs and core biopsies.

b) Competitive PCR. Competitive PCR is being performed essentially as described before. However, several modifications have been incorporated to allow for a more accurate determination in expression and add a greater level of quality control to the assay.

Densitometry - Initial quantitative measurements were being performed by laser scanning densitometry of a photographic negative. While this technology is highly accurate, the system currently available to us was cumbersome and unduly slow due to antiquated computer technology. The recent advances in computer scanning imaging and accompany imaging software has allowed densitometry to become a practical and reliable personal computing tool. We have adopted this approach, using a table top scanner, NIH Image Software, and photographic negatives to quantify our competitive assays.

Primers - In our previous report we were using primers which amplified a target region of approximately 310 bps. These primers had a low annealing temperature (38° C) and a high A/T content which could lead to non-specific priming. Furthermore, the amplified competitive template when digested with *Eco* RI generated two bands of unequal length which added complications to quantification. We are currently using nested primers internal to the previously described set, spanning nucleotides 1326-1347 and 1502-1523. These primers have a high annealing temperature (60° C), a high G/C content and amplify a target region which when cut yields two bands of equal length, thus simplifying quantification (See Figure 1, CT bands).

Restriction Enzyme Digestion - It was noted during the initial optimization of the competitive assay that, infrequently, the competitive template, at high concentrations, was digested incompletely. We have investigated the cause for this since incomplete digestion would lead to an erroneously high level of expression being reported. We initially considered that mutations were introduced into the competitive template by PCR resulting in an alteration of the restriction enzyme site. However, re-

and sequencing of the PCR product revealed no mutations.

To resolve this issue, assays were done to establish the optimal conditions for restriction enzyme digestion. The reaction buffers of typical PCRs contain salt concentrations similar to standard restriction enzyme digestions. Thus, when a restriction enzyme digestion is performed on these samples the addition of the standard restriction enzyme 10X salt buffer effectively raises the salt concentration to two-three times the recommended level for optimal activity. We have adopted a protocol were no additional 10X salt buffer is added to the reaction and only PCR product and enzyme are mixed. This protocol has resulted in approximately 100% digestion efficiency and eliminated possible errors in quantification due to incomplete digestion of amplified competitive template (Figure 1, Lane 1)

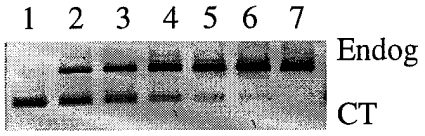


Figure 1. Competitive RT-PCR for MDR1. Competitive template (CT) is titrated in the presence of a constant amount of wild type template (Endog). Both templates are reverse transcribed and amplified simultaneously. Restriction digestion and gel electrophoresis resolves the two templates and allows for quantitative analysis by scanning densitometry as reported previously. Lane 1-400 pg CT alone; Lanes 2-6 decreasing amounts of CT and 50 ng endog RNA; Lane 7-50 ng Endog RNA alone.

Heteroduplex Formation - Heteroduplex formation is a critical factor in competitive assays using templates that are very similar or almost identical to the target gene. A PCR has three phases that are cycle and target concentration dependent. The lag phase occurs during early cycles and low target concentration. The linear amplification phase occurs during intermediate cycles and is associated with exponential increases in target. The plateau phase occurs during the latter to final cycles and is accompanied by gradual increases in target concentration. It is during the plateau phase that heteroduplex formation is most prevalent. Heteroduplexes are indigestible products and will skew the resulting titration curve toward a higher reported concentration. Our numbers recorded previously in our report are likely inaccurate secondary to heteroduplex formation. Two approaches can be used to overcome the impact of heteroduplexes in quantifying PCR products: (1) maximize heteroduplex formation and account for its formation in the final quantification calculations; or (2) optimize the quantification assay such that the PCR is measured in the linear range of amplification (LRA).

Maximization of heteroduplex formation is accomplished by amplifying the target/competitive template reaction into the plateau phase, followed by a denaturing step, 95° C for 10 minutes, to melt all the DNA duplexes. Heteroduplexes are then maximized in each reaction by allowing them to slowly cool to 4° C over 30-60 minutes. Restriction enzyme digestion and quantification is performed as reported, however the reported concentration of target sequence is multiplied by a correction factor (0.3) to account for heteroduplex formation (Becker-Andre and Hahlbrock).

An alternative approach is to prevent the formation of heteroduplexes. Heteroduplex formation is the direct result of the amplification reaction reaching a point (plateau) were the concentration of amplified target starts to greatly exceed the primer concentration. At this point the target begins to compete with the primer during the annealing step of the PCR. Thus, heteroduplexes will began to accumulate at the beginning of the plateau phase. If the reaction is limited to the LRA, the primer

concentration is essentially in greater excess than the target sequence, thus each round of amplification will see 100% of target hybridizing to primer.

We have adopted the latter approach by optimizing our reactions such that quantification occurs during the LRA. Since we use several different titration ranges of competitive template to analyze our samples, we have determined the LRA that best suits each titration range by determining the LRA for the highest concentration of competitive template used in that range (Figure 2).

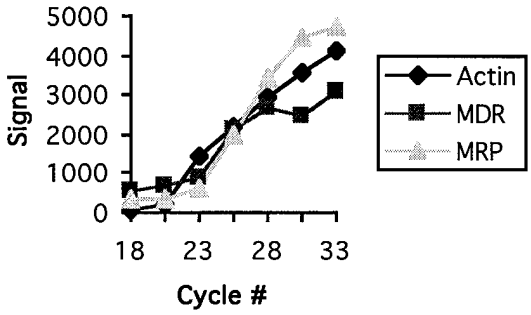


Figure 2. Linear Range of Amplification Determination. The highest concentration of competitive template used in the titration reactions for MDR and MRP were amplified using conditions as previously reported for 33 cycles. Samples were taken at various cycles and analyzed by agarose gel electrophoresis. Band intensity was measured by densitometry and plotted against cycle number. Actin was measured by analyzing the signal generated from 100 ng of cellular RNA. As can be seen , all three templates began to plateau at 28-30 cycles. Thus, with these particular titration ranges the LRA is between 22-28 cycles. Lower titration ranges have LRA between 30-34 cycles.

Absolute Concentration of Input Target RNA - One aspect of the RT-PCR assay that is subject to uncontrolled variability is the amount of target RNA added to the competitive reaction. RNA concentration is determined by standard UV spectral analysis and is subject to minor error due to the presence of various contaminants, including residual protein, DNA, and organic solvents. In addition, each sample is subject to different degrees of degradation that are not UV quantifiable and may effect absolute target concentration in each sample. Although the variability introduced may be minor and is probably insignificant in less sensitive assays, such as slot blotting, the absolute concentration is critical when using an assay where absolute molecules are being measured such as RT-PCR. One way to ensure equal loading of input target RNA is to measure an internal reference gene that is presumably expressed at equal levels in all cells, such as β-actin, GAPDH, or 18s ribosomal RNA. A simple approach is to generate cDNA for the competitive assay and reference standardization in one tube and then divide the reaction in different tubes for their respective amplifications. Samples are then compared by measuring the relative intensities of the reference standard during the LRA. This approach allows for the adjustment in absolute molecules measured based on input RNA and also allows us to directly compare different experiments.

c) Quantification of MDR1 Gene Expression - In addition to the samples on the specific clinical trials described, we have currently collected over 40 breast biopsy samples from untreated patients. These samples include ductal carcinoma *in situ* (DCIS), infiltrating ductal carcinoma (IDC), infiltrating lobular carcinoma (ILC), and normal adjacent tissue (NAT). Within this group, there are 14 sets of matched pairs, tumor and NAT. These samples have been analyzed for MDR1 gene expression as well as MRP gene expression. MRP is a recently identified multidrug resistance gene related

to MDR1 which confers multidrug resistance in cell culture when overexpressed (Cole, et al). A majority of these samples have been analyzed using our competitive assay for both genes and the results reported at the recent AACR meeting in Toronto (Abstract # 1297). This approach has allowed us to directly compare the expression of both genes. In addition, a majority of these samples (will) have extensive patient history and clinical treatment follow-up data available to make prospective correlation's.

We have analyzed the expression of both genes in a retrospective study of expression in breast tumors (Figure 3).

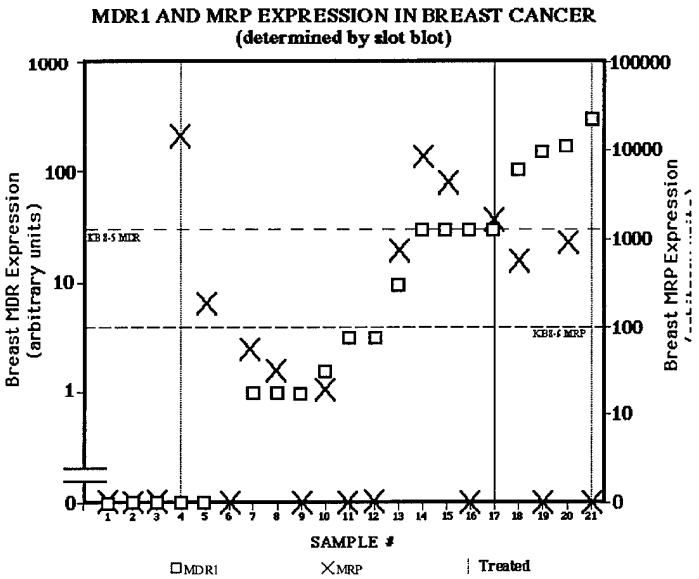


FIGURE 3. Comparison Of MDR And MRP Expression In Breast Cancer. Results obtained in slot blot analysis for *MRP* (this study) and *MDR1* (Goldstein, *et. al.* 1989. J. of NCI 81: 116-12 and this study) are graphically represented. Values are relative to the expression of KB 8-5 (MDR = 30U, MRP =100U) at 10 μg of total cellular RNA. There appears to be no correlation between the expression of MDR and MRP.

Our initial analysis indicated that some tumors had high levels of expression of one or both genes, however no clear pattern of expression was apparent. Unfortunately long-term follow-up data was not available for a majority of these samples, however it is interesting to note that, in some instances, MDR1 negative samples were positive for MRP expression which may serve as an alternative mechanism of drug resistance. Because of these findings we proceeded with competitive PCR analysis for both genes.

We have currently analyzed 27 samples be competitive PCR for both MDR1 and MRP gene expression. Figure 4 shows the level of expression for each gene.

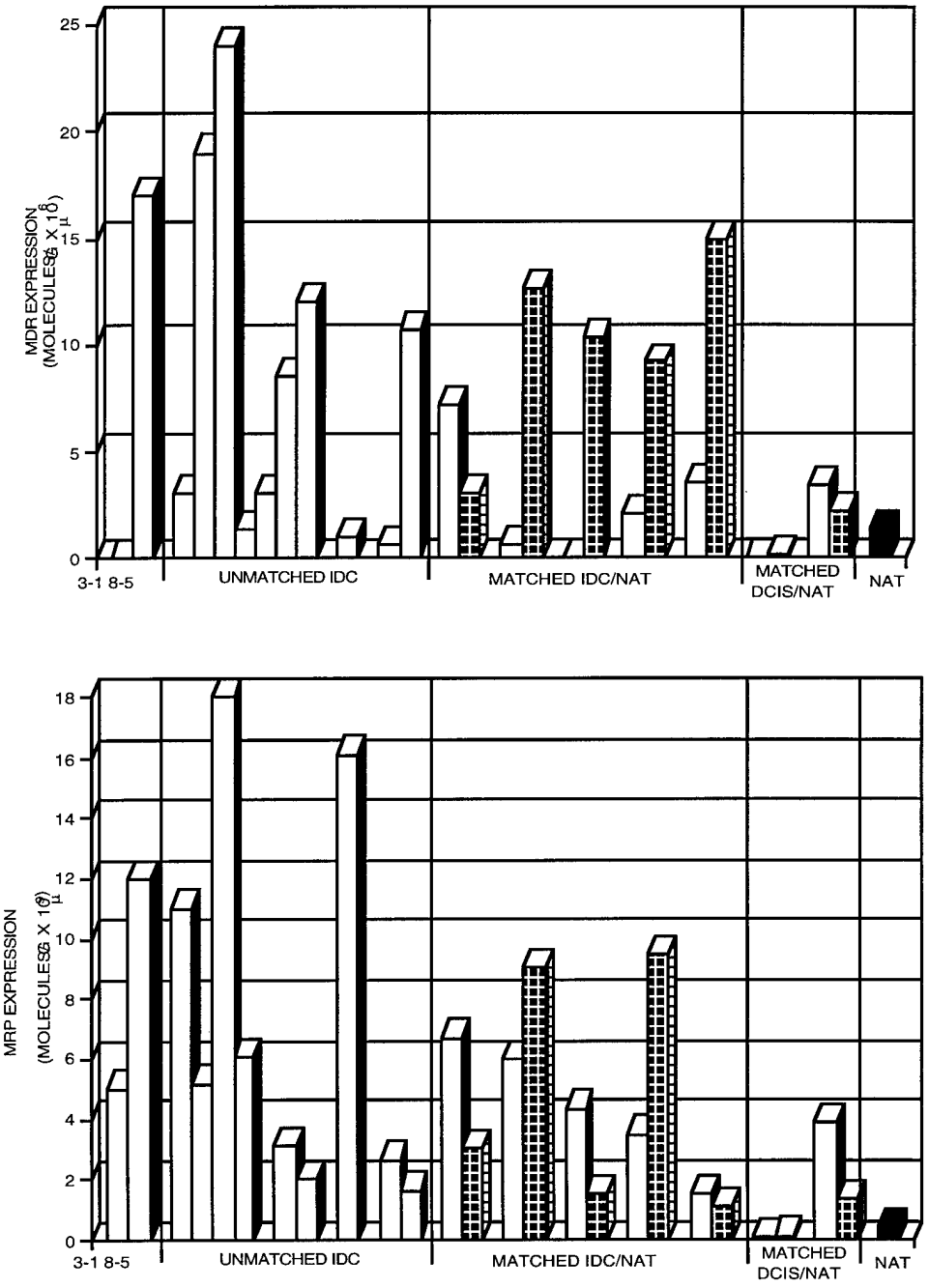


Figure 4. MDR1 and MRP gene expression in human breast cancer specimens. Expression levels were determined by competitive PCR. In several instances, normal adjacent tissue was obtained along with the tumor sample. IDC = Infiltrating Ductal Carcinoma. DCIS = Ductal Carcinoma, In Situ. NAT = Normal adjacent tissue (without matched tumor).

Of interest is the apparent higher expression of MDR1 in NAT than matched tumor. At present, insufficient samples are available to comment on the statistical significance of this difference. We are currently evaluating clinical follow-up data to determine if these levels correlate with any other known prognostic indicators or are themselves predictive of any treatment outcome.

Furthermore, we are currently evaluating MDR1 expression of these tumors (when enough RNA is available) by slot blot analysis and these data will be compared to the PCR results. However, the lack of correlation between the expression of both genes, measured by competitive PCR (Figure 5) was comparable to the data in the slot blot analysis (Figure 3).

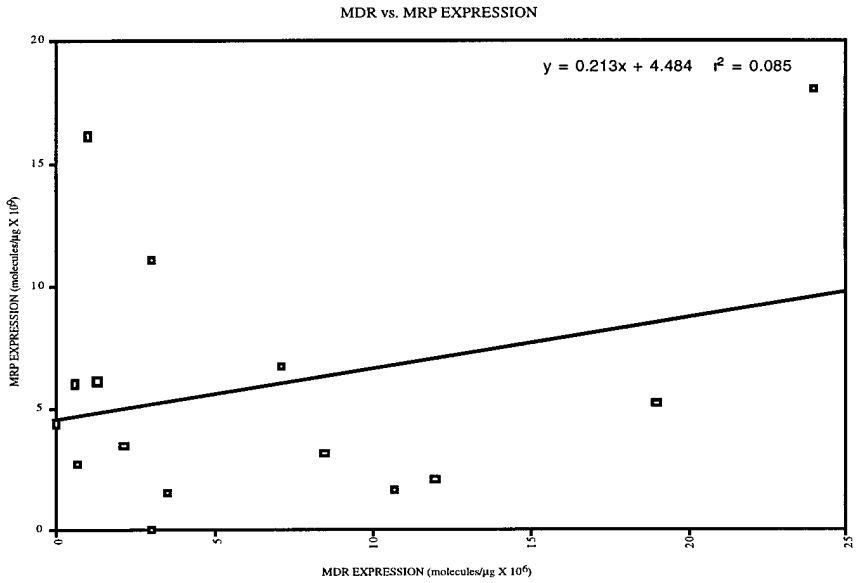


Figure 5. Correlation of Expression between MDR1 and MRP Determined by PCR.

5. Discussion

One of the major goals of this project is to determine the correlative significance of MDR1 gene expression with treatment outcome. In order to achieve this goal, the measurement of MDR1 gene expression must be reliable and consistent. There are several techniques that are currently employed to determine MDR1 gene expression levels. At issue is the reproducibility, sensitivity, specificity and quantitative nature of these methods and the correlation between them. A major focus of this study is to determine the concordance between the most common techniques (immunohistochemistry, RNA slot blot hybridization, and RT-PCR) used to measure MDR1 expression.

A considerable amount of time has been devoted to establishing appropriate procedures for each technique and evaluating the lab's consistency in performing these techniques. The sensitive nature of RNA isolation and RT-PCR require that strict protocols are followed. Thus, each technique has been scrutinized and potential pitfalls that may affect yield or introduce unwanted variability have been addressed.

RNA isolation, especially from heterogeneous tissue such as breast tumors, is extremely difficult. Furthermore, many biopsies may be from fine needle aspirates (FNAs) which can potentially contain a very finite amount of tumor. In order to assure consistent and reliable RNA extraction from human tumors as well as FNAs, we have obtained a variety of tissue biopsies (colon, liver, and breast) and perform extractions on these tissues. By extracting RNA from these samples we not only assure that our technique is consistent, we also assure the quality of our reagents.

These same samples are also analyzed by the various techniques used to assess MDR1 RNA levels. Slot blot analysis is performed using identical conditions for each blot. In order to minimize variability in hybridization several precautions are taken. Since the quality of the components of the hybridization buffers may differ from lot to lot, such as dextran sulfate, we routinely prepare, aliquots, and freeze large batches of these buffers. Each new lot is tested using standard controls before it is used for tumor work. Furthermore, fresh, unlabeled probe is prepared and quantitated for each blot. This prevents any variability in labeling due to hydrolysis of the DNA fragments. Finally, each probe is labeled to the same relative specific activity (CPM/μg).

RT-PCR is an extremely sensitive technique that requires a number of controls to be performed in order to assure the quality and validity of the reaction. In addition to the routine controls that are normally performed during RT-PCR, we also take extra precautions in terms of our competitive assay. The major concern of this assay is the loss or degradation of cRNA. In order to assure that each competitive assay receives the prescribed amount of cRNA. cRNA is quantitated by UV spectral analysis, serial dilutions are prepared, aliquoted, and stored at -80°C. Serial dilutions are only used once to avoid numerous freeze/thaw alterations and the cRNA is diluted in 10 mM Tris, 1 mM EDTA, pH 6.8. Extended RNA storage in buffers with a pH greater than 7.0 can lead to a high rate of self-cleavage. Finally, each new synthesis of cRNA is assayed against KB 8-5 to assure that its UV spectral value is equivalent to the previous cRNA. One concern that we are currently addressing is the variability in reverse-transcription of the target and cRNA sequence. We currently perform all comparative PCR assays (sample 1 versus sample 2) under concurrent conditions. Each reaction is prepared from the same master mix, same primers, and the reaction performed at the same time. This procedure eliminates any variability introduced from lot differences in reagents, i.e. dNTPs, and differences in cycling parameters. In order to assess the general efficiency of RT-PCR we have developed an assay that measures the amplification of β-actin RNA. Since each reaction contains equivalent amounts of target RNA (100 ng) the signal intensity of actin should be relatively equal for each tube. The protocol and conditions for this assay are currently being determined.

General comparisons of the various techniques will establish the relative reliability of each technique and the concordance between them. From our initial analysis it would appear that slot blot analysis and competitive PCR identified a similar trend in expression for many of the samples. In Figure 5, most breast samples that express MDR1 do so at levels less than KB-8-5 with only a few having higher expression levels which is similar to the patterns observed on slot blots. The sensitivity of slot blot hybridization does not appear to be as great as PCR. We have found the limit of resolution for RT-PCR to be no less than a two-fold difference in expression for both MDR1 and MRP. A recent study by Brophy, et. al. (1994), demonstrated that slot blot hybridization had a high false positive rate while PCR was extremely sensitive and specific supporting that MDR1 expression levels be determined utilizing RT-PCR and immunohistochemistry.

The finding of expression of both MDR and MRP in normal adjacent tissue using RT-PCR was unexpected since most previous studies have not reported expression of MDR in normal breast tissue . Wishart et al, however, has noted expression in stromal cells of breast cancer but not of normal breast. Our planned immunohistochemical studies should assist in sorting out which specific cells are PGP positive. If indeed adjacent stromal tissue stains for PGP this might suggest up regulation of MDR1 in cells at risk for tumorigenesis. It might also indicate that since MDR is a transmembrane protein, cell-cell interaction might be important for function, however, investigation of these possibilities are not within the scope of this proposal. Recent work has demonstrated that insulin like growth factor II (IGF II) is primarily stromal in origin and possibly functions as a paracrine growth promotor in breast cancer suggesting that stromal epithelial interactions may be important in breast cancer.

We are still developing immunohistochemical techniques to evaluate MDR1 expression. A panel of monoclonal antibodies, C219, JSB1, and MRK-16, are currently being used in our analysis.

The relatively ubiquitous expression of MRP compared to MDR1 has been an additionally interesting finding. Further follow-up will permit us to evaluate the patterns of co-expression

of these drug resistance genes, their correlation to drug resistance and assessment of any quantitative significance.

6. Clinical Trials

The clinical trials described in this project are at various levels of accrual, approval and development as outlined below:

a) Philadelphia Bone Marrow Consortium - PBT-3 (IRB94041). Phase II Trial of High Dose Chemotherapy with Cyclophosphamide, Thiotepa and Carboplatin and Peripheral Blood Stem Cell Infusion in Women with Inoperable Locally Advanced and Inflammatory Breast Cancer who achieved partial response to Induction Chemotherapy this protocol has been approved by the IRBs of the four member institutions including Fox Chase Cancer Center (FCCC), University of Pennsylvania, Hahnemann University and Temple University. This study was activated 6/94 and thus far, 9 patients have been enrolled and six specimens have been collected.

b) Phase I study of Cyclosporine and Quinine to Reverse MDR in Refractory Malignancy treated with Vinblastine. This study has been approved by the FCCC IRB and consent forms have already been approved by the DOD. Because Cyclosporine A (CSA) initially planned to be used in this trial has been reformulated to enhance its immunosuppressive activity and has subsequently lost its potency in mediating reversal of MDR, we are in the process of discussing with Sandoz and the NCI the possibility of substituting CSA with its analogue PSC 833 which is a more potent MDR inhibitor. When this is accomplished this study will be open for accrual.

c) Phase II Study of R-Verapamil (Dexverapamil) in Advanced Breast Cancer. This study has received FCCC IRB approval and consent forms have been approved by the DOD. R-Verapamil will likely be replaced by an alternative MDR modulator since it has not been shown to have significant clinical activity. The protocol has been written, and it has been submitted for appropriate review. The most recent draft of this protocol (E1195), a Phase II study of PSC 833 to modulate MDR mediated resistance, is enclosed and this study should be activated shortly.

d) Eastern Cooperative Oncology Group (ECOG) Registration Study of Induction with Adriamycin in Inoperable Locally Advanced and Inflammatory Breast Cancer to Evaluate for Multidrug Resistance. Since the 4/7/94 Annual Report this concept was approved by the ECOG Breast Core Committee July 7, 1994, and a draft of the schema and eligibility were included in the appendix of the previous report. An updated, completed protocol (E2195) is enclosed and this study is currently proceeding through the ECOG activation process. Both this study and PBT-3 will permit us to obtain sequential breast tumor samples before and after treatment with Adriamycin to support us in accomplishing the aims of this grant proposal i.e. to determine the clinical significance of MDR1 gene expression in breast cancer and to correlate expression with response and resistance to treatment with MDR substrate. The ECOG activation process has been delayed due to a relocation of the Operations office.

A request for an administrative extension to March 1997 has been discussed with the Contracting Officer, Mr. Brian Martin, and a letter has been forwarded to him under separate cover. This extension is requested for the necessary accrual to the clinical trials and adequate clinical follow-up to correlate clinical outcome with expression of MDR and MRP.

CONCLUSION

Drug resistance is a major obstacle in the treatment of malignancies. Although MDR1 mediated drug resistance has been well characterized in preclinical models, its role in clinical drug resistance is not as well characterized and requires further investigation. That is the aim of the studies proposed here. The ability to identify tumors with increased MDR1 gene expression has several potential applications, for example; the prediction of the response to chemotherapy or the design of studies of the reversal of resistance with agents that inhibit MDR1-mediated drug efflux. Prospective studies as described above are necessary to establish the role of MDR1 gene expression in clinical resistance. The initial goal of such trials is to demonstrate the ability

gene expression in clinical resistance. The initial goal of such trials is to demonstrate the ability to reverse MDR1 mediated drug resistance in appropriate advanced refractory malignancies. Ultimately, it will be important to incorporate these reversal strategies in the treatment of early stage disease at which time the tumor burden is smaller and fewer mechanisms of resistance may be present.

Well designed phase I and II prospective clinical trials using reversing agents in conjunction with chemotherapy in malignancies that express the MDR1 gene are necessary prior to routine use of agents such as verapamil and quinidine which carry innate toxicities. Epithelial tumors such as colon and renal cell carcinoma express the MDR1 gene and are clinically resistant to most cytotoxic agents, many of which are not substrates of P-170. In this situation, MDR may be one of a complex array of drug resistance mechanisms. Breast cancer would be a more appropriate human tumor model since it is a tumor for which many active chemotherapeutic agents are handled by MDR. In such a setting an alteration in drug efflux may indeed have an impact on response and possibly improve survival for breast cancer patients. The transgenic mouse model may be used to assess novel MDR reversing agents, non-toxic analogues of known reversing agents and combinations of various MDR modifiers to be subsequently investigated in Phase I studies. Over the period of March 15, 1993 to March 14, 1995, we have successfully outfitted our laboratory with staff, equipment, supplies and reagents to perform the necessary control experiments of MDR1 gene expression assays as described in the body of this report. We now have some preliminary data on the expression of MDR1 and MRP in breast cancer specimens and normal adjacent breast tissue. We will now follow these patients prospectively to determine the clinical significance of such expression as it relates to response and/or resistance to cytotoxins which are substrates for the proteins encoded by these genes.

REFERENCES

Becker-Andre M and Hahlbrock K: Absolute mRNA quantification using the polymerase chain reaction (PCR). A novel approach by PCR aided transcript titration assay (PATTY). Nuc Acids Res 17:9437, 1989.

Cole SPC, Bhardwaj J, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, and Deeley RV: Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 258:1650,1992.

Goldstein LJ, Galski H, Fojo A, Willingham MC, Lai S, Gazdar A, Pirker R, Green A, Crist W, Brodeur G, Lieber M, Cossman J, Gottesman MM, and Pastan I: Expression of a human multidrug resistance gene in human cancers. *J Natl Cancer Inst* 81:116,1989.

Cullen KJ, Rasmussen AA, Ellis MJ, Singer L, Hornby A, Lippman ME, Campani D, Giani C: Stromal-epithelial interactions mediated by insulin-like growth factors in breast cancer. *Proc. Am.Assoc. Cancer Res.* 36:696, 1995.

Our data show that MDR1 gene expression is important in breast cancer resistance. The role of the MDR1 gene in breast cancer treatment will be further defined by sequentially determining MDR1 gene expression pre and post treatment with doxorubicin in the context of three prospective clinical trials. In addition, this study will allow a correlation of MDR1 gene expression and clinical outcome. To determine what level of MDR1 gene expression is clinically significant, various molecular methods of determining MDR1 gene expression, including immunohistochemistry and quantitative reverse transcription followed by polymerase chain reaction, will be evaluated.

MDR can be reversed *in vitro* and we will test this hypothesis in a Phase I study of Cyclosporine A and quinine as MDR reversers of Vinblastine resistance. Together these studies will address the major goal of circumventing drug resistance in breast cancer. When the data of the MDR1 gene expression in breast cancer specimens from this proposal are available, clinical trials incorporating the modulators of MDR, Cyclosporine and quinine, will be designed for breast cancer as well. An alteration in drug efflux potentially may have an impact on response to chemotherapy and may result in improved survival for breast cancer patients. During the period between March 15, 1993 and March 14, 1995, we have outfitted our laboratory with staff, equipment, supplies and reagents, have been performing control experiments and have been pursuing activation of the various clinical trials to support this project. We now have some preliminary data on the expression of MDR1 and MRP (Multidrug resistant associated protein) in breast cancer specimens and normal adjacent breast tissue. Further follow-up of these patients is needed to determine the clinical significance of expression of these drug resistance genes.

EASTERN COOPERATIVE ONCOLOGY GROUP

A Pilot Phase II Trial of PSC 833 Modulation of Multidrug Resistance to Paclitaxol in the Treatment of Metastatic Carcinoma of the Breast

ECOG STUDY CHAIR:	Robert W. Carlson, M.D.
ECOG STUDY CO-CHAIRS:	Lorie J. Goldstein, M.D. Branimir I. Sikic, M.D.
ECOG STATISTICIAN:	Donna Neuberg, D.Sc.
ECOG PHARMACOLOGY COMMITTEE CHAIR:	Branimir I. Sikic, M.D.
ECOG BREAST COMMITTEE CHAIR:	William Wood, M.D.

STUDY PARTICIPANTS

Limited Institutions

ACTIVATION

DRAFT

INDEX

STUDY CHAIR

Robert W. Carlson, M.D.
Division of Oncology
1000 Welch Rd. Suite 202
Palo Alto, CA 94304-1808
Phone: 415/725-6457
Fax: 415/725-8222

Protocol Information Network (PIN) Contact Person

1.0 INTRODUCTION

1.1 Background

Drug resistance, which may arise by somatic mutations during tumor growth or be present de novo, is an important cause of failure of cancer chemotherapy. Most cellular models of drug resistance selected *in vitro* by anthracyclines, vinca alkaloids, or epipodophyllotoxins display a broad cross-resistance mechanism termed multidrug resistance (MDR) at the molecular and cellular level (1, 2, 3). The MDR phenotype is related to the expression of *mdr* genes coding for P-glycoproteins. P-glycoprotein acts as an efflux pump with very broad specificity and actively transports antineoplastic drugs out of the cells, thereby reducing the intracellular concentration of antineoplastic drugs.

Mdr 1 expression is strongly implicated in both intrinsic and acquired drug resistance in human cancers (4). It is likely that P-glycoprotein in normal tissues has an important role in the detoxification of certain anti-cancer agents, as well as in drug distribution as a component of the blood-brain and blood-testicular barriers (5, 6).

Several non-cytotoxic drugs, such as verapamil, phenothiazines and cyclosporines have been shown to modulate MDR, at least in part by competitive inhibition of P-glycoprotein function (3). However, verapamil and the phenothiazines modulate MDR at drug concentrations which produce unacceptable clinical toxicities (heart block and depression of the central nervous system) *in vivo.*

Cyclosporin A is a potent inhibitor of P-glycoprotein and may be safely administered in combination with etoposide, doxorubicin, or paclitaxel. Cyclosporin A does interfere with the elimination of etoposide, doxorubicin, or paclitaxel and so dose reductions are required to attain acceptable toxicities. Phase I/II trials have documented that cyclosporin A as a single agent has dose limiting toxicities of renal dysfunction and hyperbilirubinemia. Other toxicities included nausea, vomiting, increased myelosuppression, headache, hypertension, hypomagnesemia, and diarrhea.

PSC 833 is an analog of cyclosporin A, with the chemical formula [3'-keto-Bmt^{1}]-[Val^{2}]-cyclosporine. PSC 833 is highly effective at modulating multidrug resistance *in vitro* (3, 7, 8, 9). PSC 833 is superior in MDR modulation compared to amiodarone, verapamil, procaine, quinidine, quinacrine, lidocaine, and cyclosporine A. PSC 833 has produced a clear dose-dependent protective effect in mice inoculated with multidrug resistant tumors and treated with otherwise ineffective cytostatic chemotherapeutic drug doses.

A Phase I trial of PSC 833 alone followed by PSC 833 plus paclitaxel has been performed at Stanford University Medical Center. Patients were treated with paclitaxel 175 mg/m^2 as a single agent until progression of disease. Patients were then treated with PSC 833 alone for one course. Subsequent courses included PSC 833 plus paclitaxel 52.5 mg/m^2. Six cohorts of patients were treated.

Cohort 1. PSC 4 mg/kg x 2 followed by 2 mg/kg x 5 q8h.	3 patients
Cohort 2. PSC 6 mg/kg x 2 followed by 4 mg/kg x 5 q8h	3 patients
Cohort 3. PSC 8 mg/kg x 2 followed by 6 mg/kg x 5 q8h	6 patients
Cohort 4. PSC 10 mg/kg x 2 followed by 8 mg/kg x 5 q8h	3 patients
Cohort 5. PSC 6 mg/kg x 10 q6h	2 patients
Cohort 6. PSC 5 mg/kg x 10 q6h	4 patients

The dose limiting toxicity of PSC 833 was reversible ataxia (Table 1). Other toxicities that occurred included hyperbilirubinemia (2/9 patients in cohorts 3 & 4), nausea and fatigue (2/6 patients in cohorts 5 & 6). No nausea, vomiting, myelosuppression, or renal toxicity was noted with PSC 833 alone. The myelosuppression of PSC 833 plus paclitaxel 52.5 mg/m^2 was comparable to that of paclitaxel alone at a dose of 175 mg/m^2.

TABLE 1					
DOSE LIMITING TOXICITY: ATAXIA WITH PSC 833 ALONE					
		Grade			
Cohort	N of Patients	0	1	2	3
1	3	3	0	0	0
2	3	3	0	0	0
3	6	3	1	1	1
4	3	0	0	2	1
5	2	0	0	0	2
6	4	2	1	1	0

Recent phase II studies have demonstrated that paclitaxel has substantial antitumor activity in the treatment of metastatic carcinoma of the breast. In one trial of patients without prior chemotherapy for metastatic disease, a response rates of 62% was observed (10). In a trial of women with one prior chemotherapy regimen for metastatic disease a response rate of 56% was observed (11).

Paclitaxel resistance is mediated at least in part by P-glycoprotein. The reversal by PSC 833 of paclitaxel resistance in cell lines displaying the multidrug resistance phenotype has been demonstrated (7).

This phase II trial is designed to evaluate the antitumor activity of combination PSC 833 plus paclitaxel in the treatment of patients with anthracycline refractory carcinoma of the breast. The protocol should serve to address both biologic and therapeutic questions. These include an evaluation of the antitumor activity of combination PSC 833 plus paclitaxel in the treatment of breast cancer and also will provide an evaluation of the biologic activity of PSC 833 reversal of multidrug resistance.

2.0 OBJECTIVES

2.1 To evaluate the antitumor activity, as measured by frequency of objective response and time to progression, of the multidrug resistance modulator PSC 833 in combination with paclitaxel in the treatment of women with anthracycline refractory, metastatic carcinoma of the breast.

2.2 To evaluate the toxicity of combination PSC 833 and paclitaxel in the treatment of anthracycline refractory, metastatic carcinoma of the breast.

3.0 SELECTION OF PATIENTS

3.1 Patients must have a histologically confirmed, bi-dimensionally measurable, recurrent or metastatic carcinoma of the breast.

3.2 All patients must be female.

3.3 All patients must have either received prior anthracycline therapy or have a medical contraindication to anthracycline therapy.

3.4 Patients may not have received prior paclitaxel, taxotere or more than one prior chemotherapy regimen in the treatment of their recurrent or metastatic carcinoma of the breast.

3.5 No prior adjuvant chemotherapy within 6 months of diagnosis of metastatic disease (Note: prior hormonal therapy in either a metastatic or adjuvant setting is allowed.)

3.6 Patients must have an ECOG performance status of 0, 1, or 2.

3.7 Patients with central nervous system metastasis are not eligible.

3.8 Patients must have adequate bone marrow, hepatic, and renal function defined by the following:

3.81 Granulocytes $\geq$ 1500/mm^3
3.82 Platelets $\geq$ 100,000/mm^3
3.83 SGOT $\leq$ 2.5x normal
3.84 Total bilirubin $\leq$ 2x normal
3.85 Serum creatinine $\leq$ 2x normal

3.9 Patients must not have received chemotherapy or hormonal therapy for at least 3 weeks prior to enrollment.

3.10 Patients who are pregnant or lactating are ineligible. Must be using effective contraception or not be of childbearing potential.

3.11 Patients must not have had an active malignancy other than breast cancer, in situ carcinoma of the cervix, or non-melanomatous skin cancers in the past 5 years.

3.12 No active, unresolved infection.

3.13 No parenteral antibiotics 7 days prior to study entry.

3.14 No prior history of allergic reactions to drugs utilizing the vehicle Cremophor (some anesthetics and muscle relaxants).

3.15 All patients must give signed written informed consent.

4.0 REGISTRATION PROCEDURES

A signed HHS 310 Form, a copy of the institution's IRB-approved informed consent document, and written justification for any changes made to the informed consent for this protocol must be on file at the ECOG Coordinating Center before an ECOG institution may enter patients. The signed HHS 310, institution informed consent, and investigator's justification for changes will be submitted to the following address:

ECOG Coordinating Center
Frontier Science
ATTN: IRB
303 Boylston Street
Brookline, MA 02146-7648
FAX: (617) 632-2990

Patients must not start protocol treatment prior to registration.

To register eligible patients on study, the investigator will telephone the Central Randomization Desk at the ECOG Coordinating Center at (617) 632-2022. The following information will be requested:

4.1 Protocol Number

4.2 Investigator Identification

4.21 Institution name and/or affiliate
4.22 Investigator's name

4.3 Patient Identification

4.31 Patient's name or initials and chart number
4.32 Patient's Social Security number
4.33 Patient Demographics
4.331 Sex
4.332 Birthdate (MM/YY)
4.333 Race
4.334 Nine-digit zip code
4.335 Method of payment

4.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.0. An eligibility checklist has been appended to the protocol. The randomization specialist will verify eligibility by asking questions from the checklist. A confirmation of registration will be forwarded by the Coordinating Center.

4.6 Cancellation Guidelines

If a patient does not receive protocol therapy, the patient may be canceled. Reasons for cancellation should be submitted in writing to the ECOG Coordinating Center (ATTN: DATA) as soon as possible. Data will be collected on all canceled patients (see Section 10.0). Note: A patient may only be canceled if no protocol therapy is administered. Once a patient has been given protocol treatment, all forms should be submitted.

5.0 TREATMENT PLAN

5.1 Administration Schedule

Use patients actual weight when calculating body surface area.

5.11 Paclitaxel/PSC 833

PSC 833 5 mg/kg, p.o. q 6 hours x 10 doses

Paclitaxel 55 mg/m^2 IV by continuous 24 hour infusion beginning 4 hours after the 3rd dose of PSC 833

Repeat cycles every 3 weeks.

Paclitaxel will be administered over 24 hours by continuous infusion. There will be no dose escalation.

Paclitaxel must be filtered. In-line filtration with a 0.2 micron filter is required. It may be diluted in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP, 1000 ml, given over 24 hours (45 ml/hr). Paclitaxel must be prepared in glass bottles and administered with nitroglycerin administration sets (polyethylene lined PVC tubing). Treatment will be repeated every 3 weeks.

5.111 To Prevent Allergic Reactions
Due to the known toxicity or Paclitaxel and/or the Cremophor vehicle, the following precautions will be taken to decrease the possibility of anaphylaxis.

5.1111 14 hours and 7 hours prior to the Paclitaxel administration, the patient will be medicated with Dexamethasone 20 mg PO.

5.1112 30-60 minutes prior to the Paclitaxel administration, the patient will be medicated with Diphenhydramine 50 mg IV and Cimetidine (30 mg IV) or other H_2 receptor antagonist.

5.1113 Epinephrine and Diphenhydramine will be immediately available during the infusion.

5.1114 The patient's blood pressure and heart rate will be monitored during the infusion (every 15 minutes during the first hour and then every 4 hours during the remainder of the 24 hours infusion).

5.1115 See Section 5.3 for the management of hypersensitivity reactions (5.44) and cardiovascular toxicity (5.45) if they occur.

5.2 Adverse Reaction Reporting Requirements

ADR reporting should be based on the Common Toxicity Criteria (see Appendix II).

5.21 The following adverse reactions must be reported to ECOG and NCI in the manner described below. Toxicities occurring on this treatment should be considered investigational, even though the treatment contains commercial drugs as well.

Investigational Agents: PSC 833

	Grade 2-3 unexpected[1]	Grade 4 & 5 unexpected [1]	Grade 4 expected[2]	Death due to Rx or within 30 days of Rx[3]
Call to NCI within 24 hours		X		X
Call to ECOG within 24 hours		X		X
Call to drug sponsor within 24 hours		X		X
ECOG ADR Form to NCI within 10 days	X	X	X	X
ECOG ADR Form to ECOG Coordinating Center within 10 days	X	X	X	X
ECOG ADR Form to drug sponsor within 10 days		X	X	X
Notify local IRB within 10 days	X	X	X	X

1 Any unexpected toxicity not reported in the literature or the package insert must be reported.
2 Grade 4 expected myelosuppression need not be reported but should be documented on flow sheets.
3 Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.

ECOG requires ADRs to be reported on the Adverse Reaction (ADR) Form for Investigational Drugs (#391R). The form must be signed by the treating investigator.

5.22 Non-Treatment Related Toxicities

If a toxicity is felt to be outside the definitions listed above and unrelated to the protocol treatment, this must be clearly documented on the ECOG Flow Sheets which are submitted to the ECOG Coordinating Center (ATTN: DATA) according to the Records to be Kept Section (10.0). This does not in any way obviate the need for reporting the toxicities described above.

5.3 Adverse Reaction Reporting Requirements

ADR reporting should be based on the Common Toxicity Criteria (see Appendix II).

5.31 The following adverse reactions must be reported to ECOG and NCI in the manner described below.

Commercial Agents: Paclitaxel

	Grade 1-5 unexpected[1]	Death due to Rx or within 30 days of Rx[2]
ECOG ADR Form to NCI within 10 days	X	
ECOG ADR Form to ECOG Coordinating Center within 10 days	X	X
Notify local IRB within 10 days	X	X

1 Any unexpected toxicity not reported in the literature or the package insert must be reported.

2 Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.

NCI Telephone Number: (301) 230-2330
NCI FAX Number: (301) 230-0159
NCI Mailing Address:
IDB
P.O. Box 30012
Bethesda, MD 20824

ECOG Telephone Number: (617) 632-3610
ECOG Mailing Address:
ECOG Coordinating Center
ATTN: ADR
303 Boylston Street
Brookline, MA 02146-7648

ECOG requires ADRs to be reported on the Adverse Reaction (ADR) Form for Investigational Drugs (#391RF). The form must be signed by the treating investigator.

5.32 Non-Treatment Related Toxicities

If a toxicity is felt to be outside the definitions listed above and unrelated to the protocol treatment, this must be clearly documented on the ECOG Flow Sheets which are submitted to the ECOG Coordinating Center (ATTN: DATA) according to the Records to be Kept Section (10.0). This does not in any way obviate the need for reporting the toxicities described above.

5.4 Dose Modifications

All toxicities should be graded according to the Common Toxicity Criteria (see Appendix II).

5.41 Hematology Toxicity

5.411 Day 1 of each course:

Granulocytes		Platelets	Dose Paclitaxel
$\geq 1500/mm^3$	and	$\geq 10^5/mm^3$	100%
$<1500/mm^3$	or	$<10^5/mm^3$	Hold therapy until granulocytes $\geq 1500\ mm^3$ and platelets $\geq 10^5/mm^3$

If treatment is held more than 3 weeks, the patient will be taken study.

5.412 Any patient experiencing any of the following will have Paclitaxel reduced by 25%.

5.4121 Febrile neutropenic episode ($\geq$ 38.5°C) between courses.
5.4122 An absolute granulocyte count M 500/µl for $\geq$ 5 days
5.4123 Bleeding episode with a platelet count $\leq 40{,}000/mm^3$
5.4124 Platelet count $\leq$ 20,000/mm^3 with or without a bleeding episode
5.4125 Failure to recover counts for retreatment on day 22.

If, on subsequent course, these toxicities recur, paclitaxel will be reduced by another 25%.

5.42 Gastrointestinal Toxicity

5.421 Nausea and/or vomiting should be controlled with standard antiemetics.

5.422 If mucositis is present on day 1 of any cycle, the treatment should be withheld until the mucositis has cleared. If acute Grade 3 or 4 mucositis occurs, Paclitaxel should be given at 75% dose when mucositis is completely cleared for all subsequent courses.

5.423 Grade 3 or 4 diarrhea is sufficient reason to reduce the Paclitaxel dose to 75% (after resolution of diarrhea) for all subsequent courses. For grade 2, the dose should be held until the diarrhea clears, and then resumed at full dosage.

5.43 Hepatic Toxicity

SGOT	Bilirubin (mg/dl)	% Dose to give Paclitaxel
<2 x baseline	<2 x baseline	

5.44 Anaphylaxis/Hypersensitivity

5.441 Mild symptoms (e.g., mild flushing, rash, pruritus): Complete Paclitaxel infusion. Supervise at bedside. No treatment required.

5.442 Moderate symptoms (e.g., moderate rash, flushing, mild dyspnea, chest discomfort): Stop Paclitaxel infusion. Give intravenous diphenhydramine 20-25 mg and intravenous dexamethasone 10 mg. Resume Paclitaxel infusion after recovery of symptoms at a low rate, 20 ml/hr for 15 minutes, then 50 ml/hr for 15 minutes, then if no further symptoms, at full dose rate until infusion is complete. If symptoms recur, stop Paclitaxel infusion. The patient will go off study and it will be reported as an adverse event.

5.4421 Caution: Patients who had a mild to moderate hypersensitivity reaction have been successfully re-challenged but careful attention to prophylaxis and bedside monitoring of vital signs is recommended.

5.443 Severe life-threatening symptoms (e.g., hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria): Stop Paclitaxel infusion. Give intravenous diphenhydramine and dexamethasone as above. Add epinephrine or bronchodilators if indicated. The patient will go off study and it will be reported as an adverse event.

5.45 Cardiovascular

5.451 Cardiac rhythm disturbances have occurred infrequently in patients in clinical trials; however, most patients were asymptomatic and cardiac monitoring is not required. Transient asymptomatic bradycardia has been noted in as many as 29% of patients. More significant AV block has rarely been noted. Cardiac events should be managed as follows:

5.4511 Asymptomatic bradycardia: No treatment required.

5.4512 Symptomatic arrhythmia: Stop Paclitaxel and manage arrhythmia according to standard practice. The patient will off study and it will be reported as an adverse event.

5.4513 Chest pain, and/or symptomatic hypotension (<90/60 mm Hg or requires fluid replacement): Stop Paclitaxel infusion. Perform an EKG. Give intravenous diphenhydramine and dexamethasone as in 5.44 if hypersensitivity is considered. Also, consider epinephrine or bronchodilators if chest pain is not thought to be cardiac. The patient will go off study and it will be reported as an adverse event.

5.46 Peripheral Neuropathy - Neurosensory and Neuromotor

5.461 Grade 3: If grade 3 toxicity occurs, protocol treatment should be withheld until the patient recovers to grade 1 toxicity. When treatment is resumed, the

dose of Paclitaxel should be reduced by 25% and continued at this level without further reduction for neurologic toxicity.

If grade 3 toxicity persists, or if a second episode of grade 3 neurotoxicity occurs, patient should be removed form study.

5.462 Grade 4: Remove from study.

5.47 CNS Toxicities

5.471 For Grade 3 or 4 toxicity discontinue PSC 833 for current cycle and reduce PSC 833 to 4 mg/kg per dose for subsequent cycles.

5.472 If Grade 3 or 4 toxicity occurs at the reduced dose (4mg/kg) of PSC 833, the patient will go off study.

5.48 Other Toxicities

For any Grade 3 or 4 toxicity not mentioned above, treatment should be held until patients recover completely or to Grade 1 status. The next dose of Paclitaxel should be at 50% and, if well-tolerated (i.e., only Grade 1 toxicity occurs), subsequent doses should be increased by 25% in an effort to regain 100% dosing. For Grade 1 or 2 toxicities, no dose reduction should be made.

5.5 Supportive Care

5.51 All supportive measures consistent with optimal patient care will be given throughout the study.

5.52 Local radiotherapy necessary for control of pain or for life-threatening situations during the period of treatment will be considered progressive disease.

5.53 The use of G-CSF or GM-CSF for patients is allowed at the discretion of the individual investigator under established FDA guidelines.

5.6 Duration of Therapy

5.61 Patients with progressive disease or intolerable toxicity will be removed from study treatment and will be followed until death. Stable or responding disease will continue on study.

5.62 Patients with stable or responding disease and who are tolerating therapy will continue on study treatment on an indefinite basis.

6.0 MEASUREMENT OF EFFECT

6.1 ECOG Solid Tumor Response Criteria

6.11 Methods of Malignant Disease Evaluation

6.111 Measurable, Bidimensional

Malignant disease measurable (metric system) in two dimensions by ruler or calipers with surface area determined by multiplying the longest diameter by the greatest perpendicular diameter (i.e., metastatic pulmonary nodules, lymph nodes, and subcutaneous masses). Malignant disease with sharply defined borders visualized by ultrasonography or computerized axial tomography is considered measurable. Repeat studies should be performed at the same pretherapy site(s) of malignant disease.

6.1111 If liver lesions are being followed for response, there must be a baseline CT scan or MRI scan, and responses must be documented by follow-up CT or MRI scans.

6.112 Measurable, Unidimensional

Malignant disease measurable (metric system) in one dimension by ruler or calipers (i.e., mediastinal adenopathy, malignant hepatomegaly, or abdominal masses).

6.1121 Mediastinal and hilar involvement may be measured, if a preinvolvement chest x-ray is available, by subtracting the normal mediastinal or hilar width on the preinvolvement x-ray from the on-study width containing malignant disease.

6.113 Nonmeasurable, Evaluable

Malignant disease evident on clinical (physical or radiographic) examination, but not measurable by ruler or calipers (i.e., pelvic and abdominal masses, lymphangitic or confluent multinodular lung metastases, skin metastases, ascites or pleural effusions known to be caused by peritoneal or pleural metastases and uninfluenced by diuretics, liver scans, bone scans, gallium scans, deviated or obstructed ureters or gastrointestinal tract, and masses with poorly defined borders on ultrasonography or computerized axial tomography).

6.1131 Photographs should be taken prior to and during therapy to document response.

6.1132 Malignant ascites known to be caused by malignant involvement of the peritoneum and uninfluenced by diuretics may be followed by serial abdominal girths measured through a specified fixed point.

6.1133 Serial x-rays of lymphangitic or confluent multinodular lung metastases, pleural effusions, or bone metastases should be compared to evaluate response.

6.1134 Bone and neoplasm scans can be used to evaluate response.

6.1135 Chemical parameters and biologic markers will be measured during therapy, but will not be used to evaluate response, unless specifically stipulated in individual protocols.

6.2 Definitions of Response by Organ Site Involvement

6.21 Complete Response

6.211 Clinical

Complete disappearance of all clinically detectable malignant disease for at least 4 weeks. A patient who has radiographic evidence of bony metastases prior to therapy has to have normalization of radiographs or complete sclerotic healing of lytic metastases in association with a normal bone scan. A patient with an abnormal bone scan and normal radiographs prior to therapy has to have normalization of the bone scan.

6.212 Pathologic

Pathologic proof of a clinically complete response after rebiopsying areas of known malignant disease.

6.22 Partial Response

Greater than or equal to 50% decrease in tumor size for at least 4 weeks without increase in size of any area of known malignant disease of greater than 25%, or appearance of new areas of malignant disease.

6.221 Measurable, Bidimensional

Greater than or equal to a 50% decrease in tumor area (multiplication of longest diameter by the greatest perpendicular diameter), or a 50% decrease in the sum of the products of the perpendicular diameters of multiple lesions in the same organ site for at least 4 weeks.

6.222 Measurable, Unidimensional

Greater than or equal to 30% decrease in linear tumor measurement for at least 4 weeks.

6.2221 Mediastinal and hilar width response may be determined by the formula:

A = On-study width
B = Normal width (Preinvolvement x-ray)
C = Width after treatment

P.R. if $\frac{(A-B)-(C-B)}{(A-B)}$ - 0.3

6.2222 Palpable masses that can be measured in only one dimension may be evaluated for response by using the formula:

A = On-study measurement
B = Measurement after treatment

P.R. if $\frac{A-B}{A}$ - 0.3

6.2223 If liver lesions are being followed to document a partial response, there must be a baseline CT scan, and responses must be documented by follow-up CT scans.

6.223 Nonmeasurable, Evaluable

Definite improvement in evaluable malignant disease estimated to be in excess of 50% and **agreed upon by 2 independent investigators.**

6.2231 Serial evaluations of chest x-rays (i.e., confluent multinodular and lymphangitic metastases, malignant pleural effusions) and physical measurements (i.e., abdominal girth) should be documented in the records and by photograph when practical.

6.2232 The response should last for at least 4 weeks.

6.2233 A partial response of bony metastases occurs if there is a partial decrease in the size of lesions, blastic transformation of lytic lesions, or decreased density of blastic lesions, lasting for at least 4 weeks.

6.23 Stable

No significant change in measurable or evaluable disease for at least 4 weeks (greater than or equal to 12 weeks for bony metastases).

6.231 No increase in size of any known malignant disease.

6.232 No appearance of **new** areas of malignant disease.

6.233 This designation includes decrease in malignant disease of less than 50%, or decrease in unidimensional measurable disease of less than 30%, or increase in malignant disease of less than 25% in any site.

6.234 No deterioration in ECOG performance status of greater than or equal to 1 level related to malignant disease.

6.24 Progression

Significant increase in size of lesions present at the start of therapy or after a response, or appearance of new metastatic lesions **known not** to be present at the start of therapy or stable objective disease associated with a deterioration in ECOG performance status of greater than or equal to 1 level related to malignancy.

6.241 Measurable, Bidimensional and Unidimensional

6.2411 Greater than or equal to 25% increase in the area of any malignant lesions greater than 2 cm^2 or in the sum of the products of the individual lesions in a given organ site (comparison of products of the longest diameter by the greatest perpendicular diameter).

6.2412 Greater than or equal to 50% increase in the size of the product of diameters if only one lesion is available for measurement and was less than or equal to 2 cm^2 in size at the initiation of therapy.

6.2413 Greater than or equal to 25% increase in the sum of the liver measurements below the costal margins and xyphoid.

6.2414 Appearance of **new** malignant lesions.

6.242 Nonmeasurable, Evaluable

6.2421 Definite increase in the area of malignant lesions estimated to be greater than 25%.

6.2422 Appearance of **new** malignant lesions.

6.2423 Increase in size or number of bony metastases (pathologic fractures do not represent progression unless there is a documented increase in bony disease).

6.243 Nonmeasurable, Nonevaluable

Definite evidence of **new** clinically detectable (physical or radiographic) malignant disease.

6.25 No Evidence of Disease (NED)

Lack of clinically identifiable malignant disease in nonmeasurable, nonevaluable or adjuvant patients.

6.3 Evaluation of Patient's Total Response

6.31 Organ Site Evaluation

6.311 Record responses as complete (CR), partial (PR), stable (S), progression (P) or NED under appropriate methods of evaluation.

6.312 If more than one type of evaluation method exists for a given organ site, each must be recorded separately.

6.313 If there is more than one measurable lesion per organ site, an organ site PR occurs if there is a greater than 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions.

6.314 In patients with measurable disease, the worst response will prevail in determining response by organ site.

6.315 Stabilization of evaluable disease will not detract from a PR of measurable disease by organ site, but will reduce a CR to a PR.

6.316 Progression in any classification of measurability or evaluability in an organ site shall prevail as the response for that organ site.

6.32 Objective Total Patient Response

6.321 Progression occurs if any previously measurable or evaluable malignant lesions fulfill progression criteria or new malignant lesions **not known** to be present at the start of therapy develop.

6.322 Organ site stabilizations will not detract from a total patient PR in the presence of other organ site PR's and CR's.

6.323 Stabilization of evaluable disease does not detract from CR's or PR's in measurable sites, but the patient's overall response should be a PR.

6.324 Patients with a deterioration in ECOG performance status of greater than or equal to 1 level related to malignant disease are considered progressors.

6.33 Onset of Response

The time between initiation of therapy and the onset of PR or CR.

6.34 Duration of Response

Time from onset of PR or CR , whichever occurs first,(even if patient later has a CR) until objective evidence of progression.

6.35 Subjective Patient Response

In order to evaluate the quality of life during therapy, the investigator must summarize the changes in performance status and evaluate whether these changes are due to malignant disease, treatment or to unrelated factors.

7.0 STUDY PARAMETERS

a. All scans and x-rays should be done ≤ 6 weeks before registration.

b. Scans or x-rays used to document measurable disease should be done within 2 weeks prior to registration.

c. CBC with differential, LFT's should be done ≤ 2 weeks before registration.

d. All chemistries should be done ≤ 2 weeks before registration - unless specifically required on Day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to registration.

e. Hgb, Hct, WBC, Plt should be done ≤ 2 weeks before registration but if abnormal, they must be repeated < 48 hours prior to registration.

NOTE: When filling out these pre study results on the ECOG flow sheets, please make sure that ALL relevant dates are clearly given. Do NOT put all the results under the date for Day 1 of protocol treatment unless they were actually done that day. Record the actual dates.

For follow up Hgb, Hct, WBC, Plt, these tests should be done within 48 hours of the day of treatment.

	Pre-treatment	Every Cycle	Every Other Cycle	Off treatment Every 2 months
Physical Examination	X	X		X
Tumor Measurements	X	X		X
Performance Status	X	X		X
Height & Weight	X	X		X
WBC(diff), Hgb, Plt	X	X		X
Chest X-ray	X	X[1]		X
Serum creatinine	X	X		X
SGOT & Bilirubin	X	X		X
CT of Chest/Abdomen	X[2]		X[1]	X[1]
Bone Scan	X[4]			
X-rays of Bone Scan	X[3]		X[3, 5]	
Lesions or Bone Survey				

1. If lesions or abnormalities persist.
2. If clinically indicated.
3. Required only if bone scan abnormal or known lesions are present.
4. Require only if no known osseous lesions are present, with follow-up scans after 3 cycles, then every 6 months.
5. After 3 cycles, then every 2 cycles if lesions present and only site of assessable disease. Every 3 cycles if lesions present but other sites of measurable disease available to assess response.
6. All areas of measurable disease will be measured monthly unless measurements require a CT scan. If the patient has only one site of measurable disease that is documented only by CT scan, then CT scans will be required after 3 cycles then every 2 cycles thereafter. If the patient has multiple sites of measurable disease including at least one site that is not measured by CT, then the sites not requiring CT imaging will be measured monthly and sites requiring CT imaging will be imaged by CT every 3 months.

8.0 DRUG FORMULATION AND PROCUREMENT

8.1 Drug Name

PSC 833.

8.11 Classification

8.13 Mode of Action

8.14 Storage and Stability

8.15 Dose Specifics

5 mg/kg, po every 6 hours X 10 doses

8.16 Preparation

According to Sandoz the preferred way for patients to administer the PSC-833 oral solution is to add the calculated dose to a small volume of water (2 fluid ounces) and stir well. The diluted dose should be administered to the patient within 10 minutes of preparation. The medication should be taken on an empty stomach (1 hour before or two hours after meals). Water is the preferred liquid to use for dilution but orange juice, apple juice, or other non-alcoholic drinks such as soft drinks can be used. Grapefruit juice should be avoided.

8.17 Route of Administration

The oral solution should be administered on an empty stomach within 10 minutes of dilution as described above.

8.18 Incompatibilities

The oral solution should not be diluted in grapefruit juice.

8.19 Availability

PSC833 will be provided by Sandoz Pharmaceuticals.

8.110 Side Effects

1. Neurological: reversible cerebellar-dysfunction has been the dose-limiting toxicity. Symptoms that have been described include ataxia, incoordination, unsteadiness, difficulty walking. These symptoms generally appear and are most intense within 1 to 3 hours after receiving the drug. Initial data suggests that these effects are completely reversible within 12 hours of discontinuing the drug. Other neurological side effects include dizziness, a "high" feeling, diplopia, and paresthesias affecting the lips, mouth and distal extremities.

2. Gastrointestinal: Nausea, vomiting and transient increases in SGOT, SGPT and bilirubin have occurred infrequently. These side effects have been mild and reversible.

3. Cardiovascular: In one patient who developed the cerebellar side effects moderate hypertension was also documented. The hypertension recurred upon rechallenge but was reversible without any treatment.

4. Pulmonary: After intravenous (IV) administration a moderate feeling of suffocation associated with sternal pressure and an urge to cough has been reported. THESE EFFECTS HAVE NOT BEEN REPORTED TO OCCUR WITH ORAL FORMULATIONS.

8.111 Nursing Implications

1. In order to assure optimal compliance it is important to carefully instruct the patient and/or family to correctly draw up, dilute and administer their dose according to protocol instructions.

2. Patients should be informed of the likelihood of the drug causing problems with balance and coordination. **Patients should be instructed not to drive or operate potentially dangerous machinery within 24 hours of the last PSC 833 dose. Patients should also be advised to avoid alcohol, sedatives or sleeping medications while taking PSC 833 as this could increase the chances of falling.**

3. PSC 833 is a derivative of the drug cyclosporin. Cyclosporin is known to interact with many other medications. Refer to the appendix II in the protocol listing potentially significant drug interactions to assess the patient's risk for a drug interaction. While patients are taking PSC 833 they should be advised not to begin any additional medications without the knowledge of the study personnel.

8.112 References

1. SDZ PSC-833 Investigator's Brochure, September 1994.

8.2 Drug Name

Paclitaxel

8.21 Other Names

Taxol®, NSC 125973

8.22 Classification

Antimicrotubule agent.

8.23 Mode of Action

Promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell function.

8.24 Storage and Stability

The intact ampules are stored under refrigeration. Freezing does not adversely affect the product. Solutions diluted to a concentration of 0.3 to 1.2 mg/ml in normal saline or 5% dextrose are stable for up to 27 hours when stored at room temperature and normal room light. Analyses of solutions filtered through IVEX-2 and IVEX-HP (Abbott) 0.2 micron filters showed no appreciable loss of potency.

8.25 Dose Specifics

The usual dose for ovarian or metastatic breast cancer patients after failure of first-line or subsequent antineoplastic drug treatment is 135 mg/m^2 infused over 24 hours every 3 weeks. After failure of chemotherapy for metastatic breast disease or relapse within six months of adjuvant chemotherapy, paclitaxel is given at a dose of 175mg/m^2, infused over three hours and repeated every three weeks. In minimally pretreated patients, doses up to 200-250 mg/m^2 have been used, as a single agent.

8.26 Preparation

The concentrated solution must be diluted prior to use in normal saline, 5% dextrose, 5% dextrose and normal saline, or 5% dextrose in Ringer's solution to a concentration of 0.3 to 1.2 mg/ml. Solutions exhibit a slight haze, common to all products containing nonionic surfactants. Glass, polypropylene, or polyolefin containers and non-PVC-containing (nitroglycerin) infusion sets should be used. A small number of fibers (within acceptable limits established by the USP) have been observed after dilution. Therefore a hydrophilic 0.22 micron in-line filter shall be used. Analyses of solutions filtered through IVEX-2 and IVEX-HP (Abott) 0.2 micron filters showed no appreciable loss of potency. Solutions exhibiting excessive particulate formation should not be used.

8.27 Route of Administration

Usually administered as an intravenous infusion over 24 hours with a hydrophilic in-line 0.22 micron filter. One-hour intravenous infusions have been used in Phase I studies.

8.28 Incompatibilities

Avoid the use of PVC bags and infusion sets, due to leaching of DEHP (plasticizer). Prior administration of cisplatin may increase myelosuppression because of reduced clearance of taxol. Ketoconazole may inhibit taxol metabolism, based on *in vitro* data.

8.29 Availability

A concentrated solution of 6mg/ml in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol 50% is commercially available in 5 ml ampules.

8.210 Side Effects

1. Hematologic: Myelosuppression (neutropenia, leukopenia, thrombocytopenia, anemia.

2. Hypersensitivity: Thought to be caused by the Cremophor vehicle. Minor symptoms include hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, and tachycardia. More severe reactions include hypotension requiring treatment, dyspnea with bronchospasm, generalized urticaria, and angioedema. The majority (53%) of the reported reactions occurred within 2-3 minutes of initiation of treatment and 78% occurred within the first 10 minutes. Reactions usually occurred with the first and second doses.

3. Cardiovascular: Atrial arrhythmia (sinus bradycardia [usually transient and asymptomatic], sinus tachycardia, and premature beats); significant events include syncope, hypotension, other rhythm abnormalities (including ventricular tachycardia, bigeminy, and complete heart block requiring pacemaker placement), and myocardial infarction. Hypertension, possibly related to concomitant administration of dexamethasone, may also occur.

4. Neurologic: Sensory changes (taste changes); peripheral neuropathy; arthralgia and myalgia (dose-related, more common when colony-stimulating factors are also administered); seizures; mood alterations; neuroencephalopathy; hepatic encephalopathy; motor neuropathy; and autonomic neuropathy (paralytic ileus and symptomatic hypotension).

6. Dermatologic: Alopecia, universal, complete, and often sudden, between days 14-21; injection site reactions (erythema, induration, tenderness, skin discoloration); infiltration (phlebitis, cellulitis, ulceration, and necrosis, rare); radiation recall; and rash.

7. Gastrointestinal: Nausea, vomiting, diarrhea, mucositis, pharyngitis, typhlitis (neutropenic enterocolitis), ischemic colitis, and pancreatitis.

8. Hepatic: Increased SGOT (SAST), SGPT (ALT), bilirubin, alkaline phosphatase; hepatic failure, and hepatic necrosis.

9. Other: Fatigue, headaches, light-headedness, myopathy, elevated serum creatinine, elevated serum triglycerides, and visual abnormalities (sensation of flashing lights, blurred vision).

8.211 Nursing Implications

1. Monitor CBC and platelet count prior to drug administration.

2. Symptom management of expected nausea, vomiting, and stomatitis.

3. Monitor for and evaluate abdominal pain occurring after paclitaxel administration (especially in severely neutropenic patients and in those receiving G-CSF) due to the risk of ischemic and neutropenic enterocolitis.

4. Advise patients of possible hair loss.

5. Cardiac monitoring for assessment of arrhythmias in patients with serious conduction abnormalities.

6. Monitor liver function tests.

7. Advise patient of possible arthralgias and myalgias which may occur several days after treatment. Monitor for symptoms of peripheral neuropathy.

8. Monitor for signs and symptoms of hypersensitivity reactions. Insure that the recommended premedications have been given. Premedications (diphenhydramine, steroids, and H2 blocker) appear to reduce the incidence and severity of hypersensitivity reactions but do not provide complete protection. Emergency agents (diphenhydramine and epinephrine) should be available.

9. Evaluate IV site regularly for signs of infiltration. It is not known if taxol is a vesicant; however, the Cremophor vehicle for this drug can cause tissue damage.

10. In-line filtration with a 0.22 micron filter should be used.

8.212 References

1. Rowinsky EK, Casenave LA, Donehower RC. Taxol: A novel investigational microtubule agent. J Natl Cancer Inst 82:1247-1259, 1990.

2. Gregory RE, DeLisa AF. Paclitaxel: A new antineoplastic agent for refractory ovarian cancer. Clin Pharm 12:401-15, 1993.

3. Rowinsky EK, et.al. Clinical toxicities encountered with paclitaxel. Semin Oncol 20:1-15, 1993.

4. Walker FE. Paclitaxel: Side effects and patient education issues. Semin Oncol Nurs 9(suppl 2):6-10, 1993.

9.0 STATISTICAL CONSIDERATIONS

This study seeks to assess the efficacy of paclitaxel plus PSC 833 in patients with anthracycline resistant metastatic breast cancer. Paclitaxel alone, as a single agent, is reported to be associated with a 56% objective response rate with patients who have received one prior chemotherapy for their breast cancer. Our hypothesis is that in patients with anthracycline resistant disease, the addition of PSC 833 can permit that same single agent response rate to be achieved in these anthracycline-resistant patients.

We propose a two stage design for this trial. In the first stage, 17 patients would be entered on study with the expectation that 15 would be eligible. If six or more objective responses are observed among these 15 patients, an additional 15 patients would be accrued to the study. The treatment would be considered worthy of further investigation if at least 14 of these 30 patients exhibit objective responses to therapy. If the true objective response rate for paclitaxel plus PSC 833 in this patient population is indeed 55%, the probability that the study will stop early is 0.08, and the overall probability of concluding that this regimen merits further study, or the power to detect a 55% response rate, is 0.84. If on the other hand, the true response rate to paclitaxel plus PSC 833 is 40%, the probability of stopping the study early is 0.40, and the probability of incorrectly concluding that the regimen should receive further study is 0.26.

We anticipate that a total of 34 patients could be accrued to this protocol by ECOG within 24 months.

10.0 RECORDS TO BE KEPT

The following forms must be submitted to the ECOG Coordinating Center, Frontier Science, 303 Boylston Street, Brookline, MA 02146 (ATTN: DATA).

Form	To Be Submitted
* _____ On-Study Form	Within one week of registration
ECOG CTC Flow Sheet	Every (month/3 months) while on treatment
ECOG Measurement Form	
ECOG Follow-Up Form Parts A, B, C, D, E	Every (month/3 months) while on study treatment and at completion of treatment
* Parts A, B	Off Treatment: • every 3 months if patient is <2 years from study entry • every 6 months if patient is 2-5 years from study entry • every 12 months if patient is > 5 years from study entry
Adverse Reaction (ADR) Form for Investigational Drugs (#391RF)	Within 10 days of reportable event as defined in Section 5.2.

* These forms are to be submitted for all canceled patients according to the above schedule.

11.0 PATIENT CONSENT AND PEER JUDGMENT

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

12.0 REFERENCES

1. Pastan I, Gottesman M. Multiple-drug resistance in human cancer. New England Journal of Medicine 1987;316:1388-1393.

2. Goldstein LJ; Pastan I; Gottesman MM. Multidrug resistance in human cancer. Critical Reviews in Oncology/Hematology, 1992, 12(3):243-53.

3. Sikic BI. Modulation of multidrug resistance: at the threshold [editorial; comment]. Journal of Clinical Oncology, 1993 Sep, 11(9):1629-35.

4. Goldstein LJ, Galski H, Fojo A, *et al.* Expression of a multidrug resistance gene in human cancers. Journal of the National Cancer Institute 1989;81:1116-1124.

5. Thiebault F, Tsuruo T, Hamada H, *et al.* Cellular localization of the multidrug-resistant gene product P-glycoprotein in normal human tissues. Proceedings of the National Academy of Sciences, USA 1987;84:7735-7738.

6. Cordon-Cardo C, O'Brien JP, Casals D, *et al.* Multidrug-resistance gene (P-glycoprotein) is expressed by endolthelial cells at blood-brain barrier sites. Proceedings of the National Academy of Sciences, USA 1989;86:695-698.

7. Jachez B, Nordmann R, Loor F. Restoration of paclitaxel sensitivity of multidrug-resistant cells by the cyclosporine SDZ PSC 833 and the cyclopeptolide SDZ 280-446. Journal of the National Cancer Institute, 1993, 85:478-83

8. Boesch D, Gaveriaux C, Jachez B, *et al.* In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Research 1991;51:4226-4233.

9. Twentymann PR, Bleehen NM. Resistance modification by PSC-833, a novel non-immunosuppressive cyclosporin [corrected] [published erratum appears in European Journal Cancer 1992;28:616]. European Journal of Cancer 1992;27:1639-1642.

10. Reichman BS, Seidman AD, Crown JP, *et al.* Paclitaxel and recombinant human granulocyte colony-stimulating factor as initial chemotherapy for metastatic breast cancer. Journal of Clinical Onclogy 1993;11:1943-1951.

11. Holmes FA, Valero V, Walters RS, *et al.* The M.D. Anderson Cancer Center experience with Taxol in metastatic breast cancer. Monographs / National Cancer Institute 1993;15:161-169.

DRAFT July 9, 1995 E2195

EASTERN COOPERATIVE ONCOLOGY GROUP

Induction with Adriamycin in
Inoperable Locally Advanced and Inflammatory Breast Cancer to
Evaluate for Multidrug Resistance
A Registration Study

STUDY CHAIR:	Lori J. Goldstein, M.D.
STUDY CO-CHAIR:	
MODALITY CO-CHAIR(S):	
STATISTICIAN:	Donna Neuberg, DSc.
(MODALITY ORIENTED) COMMITTEE CHAIR:	
BREAST COMMITTEE CHAIR:	William Wood, M.D.

STUDY PARTICIPANTS
ECOG Entire Group

Intergroup

ACTIVATION DATE
Draft

INDEX

STUDY CHAIR

Lori J. Goldstein, MD
Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia , PA 19111
Phone: (215) 728-2689
FAX: (215) 728-4333

Protocol Information Network (PIN) Contact Person

Amy Kramer, MRN
Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia, PA 19111
Phone: (215) 728-2975
FAX: (215) 728-2687

SCHEMA

ECOG 2195
Induction with Adriamycin in
Inoperable Locally Advanced and Inflammatory Breast Cancer to
Evaluate for Multidrug Resistance
A Registration Study

Stratification	REGISTER		EVALUATE		
Disease: Locally Adv. Inflammatory Menopausal Status:Pre- or Post ER Status: positive or negative		Diagnostic Biopsy for Molecular analysis of Drug Resistance[1] THEN Adriamycin(doxorubicin) 30 mg/m^2 IV Day1,2,3 Repeat every 3 weeks X 4		Surgery Mastectomy or Incisional or Core Biopsy Specimen analyzed for molecular analysis of Drug Resistance[1]	Further treatment with Radiation and/or Chemotherapy to be determined by individual investigator

1 Biopsies (Core , Incisional or fine needle aspirates*) and representative portions of the mastectomy specimens (optimally 500-1000 mg or 1 cm^3) will be frozen in liquid nitrogen or dry ice and stored at -70 ° C and shipped on dry ice to:

Lori J. Goldstein, M. D.
Department of Medical Oncology
Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia, PA 19111

* Fine Needle Aspirate Procedure: A minimum of two, and optimally three Fine Needle Aspirates(FNA) are required, one for cytology to confirm diagnosis and two for MDR analysis. Diagnosis will be done at the treatment site and the report will be sent to the data manager. Lysis buffer will be provided for on-site sample suspension. The FNA can be drawn and simply injected into the lysis buffer which consists of a quanidinium isothiocyanate salt solution. The sample can then be stored at -20°C and shipped on wet ice. Otherwise, the FNA should be stored in the syringe/ needle apparatus at -70°C and shipped overnight on dry ice.

2 Use the patient's actual weight when calculating surface area.

1.0 INTRODUCTION

Breast cancer represents the most common malignancy in women. It is estimated that in 1993, 182,000 new cases of invasive breast cancer will be diagnosed in the USA. Approximately 20% of breast cancers at the time of presentation are locally advanced without evidence of distant metastases and two-third of these are classifies as locally advanced inoperable or inflammatory breast cancer[1]. In general, locally advanced inoperable breast cancer is categorized by tumors of any size with chest wall fixation, edema. skin ulceration or satellite nodules, the presence of fixed ipsilateral lymph nodes or arm edema. Previously, ipsilateral supraclavicular nodes were categorized as stage III, however, they are now classified as metastatic disease and therefore, constitute stage IV disease. The guidelines for operability were originally described by Haagensen and Stout (2). Inflammatory breast cancer represents a distinct clinical entity characterized by the presence of diffuse erythema, increased warmth of the involved skin, edema of the skin (peau d'orange) and induration of the underlying breast tissue with or without a palpable mass (3,4,5). The hallmark pathologically is the presence of dermal lymphatic invasion, however, pathologic confirmation of dermal lymphatic invasion is not required for a diagnosis of inflammatory breast cancer since multiple series demonstrated no difference in survival rates between patients with a clinical diagnosis that those with a clinical and pathologic diagnosis (3,6,7,8,9,10).

Historically, locally advanced inoperable breast cancer and inflammatory breast cancer were treated with radiotherapy alone. Employing doses of 6000 to 9000 cGy, local-regional recurrence rates ranged from 30-60% and 5-year survival ranged from 5 to 40% (11,12,13,14,15,16). With local-regional treatment alone, 80 to 90% of patients developed distant metastases (11,12,13).

Induction or neoadjuvant chemotherapy has become standard treatment in patients with locally advanced inoperable or inflammatory breast cancer in an attempt to decrease the risk of distant metastasis as well as to assess the response of the primary tumor to treatment. Multiple regimens have been employed to treat these patients. The studies are difficult to compare and no direct randomized trial has evaluated Doxorubicin versus non-Doxorubicin based combinations. It does appear, however, that some conclusions can be reached. Using Doxorubicin-based combinations, response rates of over 80% have been consistently seen with complete responses ranging from 10 to 49% (17-26). For inflammatory breast cancer, similar complete response rates have been reported (27,28). Five-year survival employing induction chemotherapy with radiotherapy with or without mastectomy ranges from 40- to 60% with a disease-free survival of 30 to, (29,30). Regimens employing estrogenic recruitment and hormonal synchronization have increased the comlete response rate to 50% for stage IIIB and inflammatory breast cancer, however, the 5-year survival remains in the same range of 25% to 30% (10,26). A general conclusion from these studies is that those patients who achieve a clinical and pathological complete response tend to have longer overall survivals. Our goal is to elucidate some of the mechanisms for intrinsic and acquired drug resistance to potentially improve induction regimens for these patients.

The optimal induction regimen in either locally advanced or inflammatory breast cancer has not been well defined. As previously stated, one can draw the conclusion that a doxorubicin-containing regimen may be superior to those not including the anthracycline. However, to give this as a single agent or in combination and the optimal dose and schedule have not been established by randomized studies. Recently some reports have stressed that the use of high -dose or intensive doxorubicin may be superior to standard dose. Jones et al reported an 85% response rate with intensive dose doxorubicin and a clinical CR of 38%. Based on these studies, ECOG and CALGB completed a study of intensive doxorubicin and the preliminary data indicate at least a 75% response 50% (10,11,14,18,20,27) rate and 19% CR in locally advanced and inflammatory breast cancer and we propose to use this dose and schedule of doxorubicin in this study.

Part of the problem faced in improving the plight of patients with breast cancer has been the lack of active new agents. Experience with other diseases (e.g., testicular cancer) has suggested that the addition of a single new active agent to other existing agents may result in major improvements in patient survival, with the potential for cure of widespread disease.

1.1 Multidrug Resistance in Breast Cancer

Drug resistance is a major obstacle in the treatment of cancer. The multidrug resistance gene (MDR1) encodes an energy dependent drug efflux pump, P-170, that confers cellular resistance to multiple therapeutic agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins, taxol, and actinomycin-D (31-34). MDR1 gene expression is tumor specific in both de novo resistant tumors and those that acquire drug resistance following chemotherapy (35). The central role of P-170 in this MDR phenotype suggests that modulation of either MDR1 gene expression or the function of P-170 may provide an effective means of clinically reversing drug resistance.

1.1 1 Although there is a significant response rate to doxorubicin in breast cancer, resistance and relapse are the usual outcome in advanced disease. The role of MDR in breast cancer has been studied by several investigators as seen in Table 1.

TABLE 1: MDR1 EXPRESSION IN BREAST CANCER

STUDY	METHOD	UNTREATED	TREATED*
Goldstein et al. (35)	RNA Slot Blot, RNase Protection Assay	9/57 (16%)	5/7 (71%)
Wallner et al. (36)	RNA Slot Blot	17/59 (29%) low 10/59 (17%) high	
Keith et al. (37)	RNA Dot Blot Northern Blot	25/49 (51%)	
Merkel et al. (38)	Southern, Northern, or Western Blot	0/219	0/29
Schneider et al. (39)	Immunohistochemistry C219	2/12 (16%)	3/4 + (non-MDR drugs)
Wishart et al. (40)	Immunohistochemistry C219**, MRK 16 ∴	21/29 ++ (72%) 16/29 ∴ (55%)	
Ro et al. (41)	Immunohistochemistry C219	0/8	
Verrelle et al. (42)	Immunohistochemistry C494	17/20 (85%)	
Sugawara et al. (43)	Immunohistochemistry MRK 16	1/9 (11%)	
Gerlach et al. (44)	Western Blot C219, C494	0/3	
Sanfilippo et al. (45)	Immunohistochemistry C219	10/34 (29%)	9/14 (64%)

* Samples not obtained sequentially pre- and post-treatment in the same patients.
\+ isolated tumor cells identified as MDR-1 positive.
++ ≥20%tumor cells identified as MDR-1 positive.

The results of previously published studies of the role of the MDR1 gene in human malignancies are limited in several ways. A detailed analysis of the results from several studies of MDR1 gene expression in breast cancer from Table 1 is an illustration of these points. Many early studies were limited by small sample size which is illustrated by Ro, Sugawara and Gerlach whose studies demonstrate little to no MDR1 gene expression but the denominator is too small to reach any conclusion about the significance of the results (41,43,44). In addition, most of these studies except that of Wallner and Verrelle are retrospective analyses done on archival frozen tissue without any clinical correlation (36,42). This may result in possible selection bias. Verrelle et al, used an anti P-170 monoclonal antibody C494 in an avidin-biotin-immunoperoxidase technique and detected P-170 in 17 of 20 breast cancer specimens (42). The authors used a semi-quantitative method of analysis by grading both the number of positive cells and the specific staining intensity. Though the numbers of patients in this study was limited, strong P-170 positive staining was found in the majority of tumor cells significantly correlated with no initial response to chemotherapy ($p<.02$) and with a shorter progression free survival ($p<.02$). Further follow-up is needed to determine if Wallner's results from primary breast cancer specimens have any prognostic significance (36).

Another major limitation of these studies is the absence of sequential tumor sampling before and after treatment with a cytotoxic agent which is a substrate of P-170. Such sampling would enable us to delineate the role of MDR mediated intrinsic and acquired drug resistance in breast cancer.

Issues of method selection for detection of MDR1 gene expression (RNA vs. protein; quantitative vs. qualitative assays); selection of a definition of a positive control which would affect the sensitivity of the method chosen and the sensitivity and specificity of the given cDNA probes and monoclonal antibodies (MoAb) may all contribute to the disparate results noted in the untreated breast cancer studies.

Definition and significance of a positive result using immunohistochemistry may contribute to the inflated results seen in those studies using immunohistochemistry where most authors use any positive cells as a positive result. For example Schneider's result of 0 of 12 could be reinterpreted as 2 of 12 if any staining were considered positive as in the Wishart study (39,40). The major difference between immunohistochemical techniques and RNA analysis include measuring protein as opposed to RNA, however, neither method assesses protein function. Immunohistochemistry also has the advantage of analyzing individual cells such that tumor cell expression can be differentiated from adjacent normal cells and stroma whereas isolating RNA from a solid tumor lacks such discrimination. Of importance however is that Wishart recently reported that P-170 expression was noted in stromal cells in breast cancer but not those of normal breast tissue (46). Although immunohistochemistry is capable of detecting low levels of expression in individual cells, at the lower limit it may be incapable of distinguishing true expression from background and in that respect may not be as sensitive as detection of MDR1 RNA using PCR. In addition because of the heterogeneity of staining, immunohistochemistry is not as quantitative as RNA slot blots.

The other major factor is the sensitivity and specificity of the specific monoclonal antibody (MoAb) used. As noted, Schneider found no expression of P-170 (39). Verrelle detected expression in 85% and found different results with different antibodies (42). The most commonly used MoAbs are C219 and MRK16. Others include C494, JSB-1 and HYB-241. C219, C494 and JSB-1 recognize internal epitopes while the others recognize external epitopes. Specificity is a significant issue in that C219 cross reacts with MDR3, which has not been demonstrated to confer drug resistance, and with myosin. MRK16 is specific for MDR1 but may have heterogeneous staining even in control cell lines. Differences in fixation techniques may also contribute to the variability in results even when the same antibody is used.

The aim of this proposal is to resolve these issues by prospectively analyzing breast cancer

specimens before and after treatment with Adriamycin and correlating response or resistance to MDR1 gene expression. We will also compare the various methods of measuring MDR1 gene expression with each other.

1.3 Adriamycin (Doxorubicin)

The anthracycline Adriamycin has been used for the treatment of metastatic breast cancer for the past two decades, where it has proven itself to be an active agent both as first- and second-line chemotherapy for metastatic breast cancer. Adriamycin works as a DNA intercalator, and in breast cancer there is evidence for a dose-response effect. Both the peak concentration and AUC of doxorubicin and its major metabolite, doxorubinicol, increase as administered bolus dose increase, without any significant change in terminal half-life (47). Trials of Adriamycin used in increased doses as a single agent in breast cancer patients have shown it to have relatively greater activity than when used in lower doses. Brambilla et al demonstrated an overall response rate to Adriamycin at 75 mg/m^2 of 52% in a group of patients who had received either no prior chemotherapy (6/8 responses, 75%) or one prior chemotherapy (5/13 responses, 38%) (48). Bronchud et al showed an overall response rate of 80% in breast cancer patients receiving >75 mg/m^2 of Adriamycin as initial chemotherapy for their disease (49). Carmo-Pereira et al demonstrated an overall response rate of 58% in 24 previously untreated patients receiving 70 mg/m^2 of Adriamycin, comparable to 57% response rate reported by Steiner et al in previously untreated patients with metastatic breast cancer (50,51).

The toxicity of doxorubicin administered at 75 mg/m^2 has been evaluated by Brambilla *et al* (48). Though neutropenia was common, infections were rare; thrombocytopenia was never dose-limiting. A specific and transient EKG abnormalities (tachycardia, premature ventricular beats, ST-T segment depression, and flattening and inversion of T waves) were documented in 18% of patients. As in other studies, declines in ejection fraction were seen with prolonged administration. Similar toxicities were seen when Adriamycin was administered at 70 mg/m^2 (50-51).

1.5 Significance of Proposal

Our data show that MDR1 gene expression is important in breast cancer resistance. The role of the MDR1 gene in breast cancer treatment will be further defined by sequentially determining MDR1 gene expression pre- and post-treatment with Adriamycin or Taxol in the context of the active ECOG Phase III trial of Adriamycin vs. Taxol vs. Adriamycin-Taxol in Metastatic Breast Cancer (E1193). In addition, this study will allow a correlation of MDR1 gene expression and clinical outcome. To determine what level of MDR1 gene expression is clinically significant, various molecular methods of determining MDR1 gene expression, including immunohistochemistry and quantitative reverse transcription followed by polymerase chain reaction, will be evaluated.

Drug resistance is a major obstacle in the treatment of malignancies. Although MDR1-mediated drug resistance has been well characterized in preclinical models, its role in clinical drug resistance is not as well characterized and requires further investigation. That is the aim of the study proposed here. The ability to identify tumors with increased MDR1 gene expression has several potential applications; for example, the prediction of the response to chemotherapy or the design of studies of the reversal of resistance with agents that inhibit MDR1-mediated drug efflux. Prospective studies as described above are necessary to establish the role of MDR1 gene expression in clinical resistance. Breast cancer is an appropriate human tumor model for studying the role of the MDR1 gene since it is a tumor for which many active chemotherapeutic agents are handled by MDR. In such a setting an alteration in drug efflux may indeed have an impact on response and possibly improve survival for breast cancer patients. While Adriamycin is currently the most active drug in advanced breast cancer, the introduction of Taxol, another substrate of P-170, into the clinical armamentarium of breast cancer treatment also requires a need for

understanding its resistance. The subsequent initial goal of such studies is to demonstrate the ability to reverse MDR1 mediated drug resistance in appropriate advanced refractory malignancies. Ultimately, it will be important to incorporate these reversal strategies in the treatment of early stage disease at which time the tumor burden is smaller and fewer mechanisms of resistance may be present.

2.0 OBJECTIVES

The major goal of this clinical laboratory study is to evaluate the role of MDR1 gene expression in Adriamycin resistance Stage III breast cancer patients undergoing induction (neoadjuvant) treatment with Adriamycin. The strategy to accomplish the objective of this study is:

2.1 Correlate MDR1 gene expression with response and resistance to doxorubicin.

2.2 Correlate clinical and pathological response to induction chemotherapy with disease free and overall survival.

2.3 Correlate MDR1 gene expression with disease free and overall survival.

3.0 SELECTION OF PATIENTS

3.1 Histologically or cytologically documented clinical Stage III adenocarcinoma of the breast, any T with N_2 or N_3, $M_0T_3N_1$, M_0T_4 any N, M_0. Stage to is to be determined according to updated AJC staging system (see appendix. Note that T_3N_0 tumors are now considered Stage IIB and are not eligible.

3.2 Measurable or evaluable disease confined to the breast and regional lymph nodes. Synchronous bilateral primary cancers if Stage III may be included.

3.21 Measurable disease: any clearly defined mass or lymph node measuring $\geq$ 2cm on physical examination, sonography, CT scan or mammogram.

3.22 Evaluable disease: any poorly circumscribed mass or inflammatory cancer that cannot be reproducibly measured on physical examination, sonography, CT scan or mammogram.

3.23 All patients must be evaluated by a radiation oncologist before induction chemotherapy begins.

3.3 Prior Therapy

3.31 No prior therapy apart from biopsy.

3.32 Patients having excisional biopsy are excluded.

3.33 No previous chemotherapy or hormonal therapy for breast cancer. Hormonal therapy given for menopausal symptoms does not disqualify patients.

3.34 No prior doxorubicin.

3.35 No prior radiation to the proposed treatment area.

3.36 No clinical or imaging evidence of distant metastasis.

3.4 Expected survival > 6 months

3.5 Age ≥ 18

3.6 ECOG Performance status: 0 - 1

3.7 Non-pregnant, non-lactating

3.8 No previous breast cancer.

3.9 Required Initial Parameters

Granulocytes	≥ 1500/µl
Platelet count	≥100.000/µl
Hemoglobin	≥ 10 gm/dl
BUN	< 1.5 x normal
Creatinine	< 1.5 x normal
Bilirubin	normal
Albumin	normal
Left ventricular ejection fraction	normal
ER and PgR	Known or pending; biochemical assay or immunohistochemistry acceptable.

Pre-chemotherapy CT scan of chest is required
Photographs of involved region for patients with inflammatory breast cancer.

3.10 All patients registered must have fresh biopsies of the involved breast or lymph nodes.

Biopsies of tumors from patients described in this study will be fresh frozen in liquid nitrogen or dry ice within 2 hours of biopsy without preservatives or fixatives. Specimens should be stored at -70° C in sterile nonglass containers and shipped on dry ice in a styrofoam container to: Lori J. Goldstein, M.D., Department of Medical Oncology, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111. **(Please refer to Appendix II for instructions regarding specimen collection and shipping procedures.)**

3.11 Signed written informed consent.

3.12 No previous or concomitant malignancy is allowed, except inactive non-melanoma skin cancer , in situ carcinoma of the cervix or other non-breast cancers if the patient has been disease free for ≥10 years.

3.13 No other serious medical or psychiatric illness which would prevent informed consent.

3.14 No history of congestive heart failure, myocardial infarcton within 6 months, or symptomatic cardiac arrhythmias requiring medication.

3.15 No history of chronic liver disease.

4.0 REGISTRATION PROCEDURES

A signed HHS 596 or 310 Form, a copy of the institution's IRB-approved informed consent document, and written justification for any changes made to the informed consent for this protocol must be on file at the ECOG Coordinating Center before an ECOG institution may enter patients. The signed HHS 596, institution informed consent, and investigator's justification for changes will be submitted to the following address:

ECOG Coordinating Center
Frontier Science
303 Boylston Street

Brookline, MA 02146-7648
FAX 617/632-2990

Patients must not start protocol treatment prior to registration.

To register eligible patients on study, the investigator will telephone the Central Randomization Desk at the ECOG Coordinating Center at (617) 632-2022. The following information will be requested:

4.1 Protocol Number

4.2 Investigator Identification

4.21 Institution name and/or affiliate

4.22 Investigator's name

4.3 Patient Identification

4.31 Patient's name or initials and chart number

4.32 Patient's Social Security number

4.33 Patient Demographics

4.331 Sex

4.332 Birthdate (MM/YY)

4.333 Race

4.334 Nine-digit zip code

4.335 Method of payment

4.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.0. An eligibility checklist has been appended to the protocol. The randomization specialist will verify eligibility by asking questions from the checklist. A confirmation of registration will be forwarded by the Coordinating Center.

4.5 Cancellation Guidelines

If a patient does not receive protocol therapy, the patient may be canceled. Reasons for cancellation should be submitted in writing to the ECOG Coordinating Center (ATTN: DATA) as soon as possible. Data will be collected on all canceled patients (see Section 11.0). Note: A patient may only be canceled if no protocol therapy is administered. Once a patient has been given protocol treatment, all forms should be submitted.

5.0 TREATMENT PLAN AND METHODOLOGY

5.1 Processing of Patient Specimens for Submission and Subsequent Tissue Analysis

5.11 We plan to sequentially evaluate MDR1 gene expression in breast cancer pre and post treatment with Adriamycin or Taxol. Specimens from patients with biopsiable Stage III breast cancer will be obtained from patients entered on this study prior to induction treatment with Adriamycin. After induction chemotherapy patients will undergo either a radical mastectomy, modified radical mastectomy, incisional biopsy

or core biopsy depending upon the response to therapy. At both timepoints biopsies will be obtained, stored, mailed and processed as described below.

5.12 For patients registered on protocol E7194 with biopsiable Stage III Breast Cancer, biopsies will be obtained prior to induction treatment and after response to induction therapy has been assessed.

5.13 Specimens at the first time point prior to therapy will be obtained by incisional biopsy when accessible, in some cases by core biopsy and less optimally by *Fine Needle Aspirate (FNA). Optimally 500-1000 mg of tissue (minimal amount of tissue of 0.5 x 0.5 cm) or 3 core biopsies will be required for analysis.

5.14 Biopsies of tumors from patients described in this study will be fresh frozen in liquid nitrogen or dry ice within 2 hours of biopsy without preservatives or fixatives. Specimens should be stored at -70°C in sterile nonglass containers and shipped on dry ice in a styrofoam container to: Lori J. Goldstein, M.D., Department of Medical Oncology, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111. **(Please refer to Appendix II for instructions regarding specimen collection and shipping procedures.)**

5.15 * **Fine Needle Aspirate Procedure:** A minimum of two, and optimally three Fine Needle Aspirates(FNA) are required, one for cytology to confirm diagnosis and two for MDR analysis. Diagnosis will be done at the treatment site and the report will be sent to the data manager. Lysis buffer will be provided for on-site sample suspension. The FNA can be drawn and simply injected into the lysis buffer which consists of a quanidinium isothiocyanate salt solution. The sample can then be stored at -200 C and shipped on wet ice. Otherwise, the FNA should be stored in the syringe/needle apparatus at 70°C and shipped overnight on dry ice.

5.2 Analysis of MDR1 Gene Expression

MDR1 gene expression will be measured by 4 methods, including immunohistochemistry, RNA slot blot, RNase protection assay and RT-PCR. When adequate tissue is available all methods will be done on each specimen. Expression by each method will be compared to each other and to clinical drug resistance. Although large numbers of patient samples would be required to provide statistical significance, a detailed approach such as this should contribute to answering the question of what level of MDR1 expression is clinically significant as methods of detection become more sensitive. Differences in results between these methods may lead to future experiments concerning transcriptional and translational control.

5.21 Immunohistochemistry

A portion of each specimen will be pathologically analyzed and then evaluated for immunohistochemical stains. Immunohistochemistry will be used to measure MDR-1 gene expression at the protein level. Immunohistochemistry has the advantage over RNA analysis of being cell specific and therefore may overcome tissue heterogeneity. Immunohistochemical studies will use MDR-1 monoclonal antibodies, MRK16, C219 and JSB1 and be done in collaboration with Jose Russo, M.D., Chairman, Department of Pathology, Fox Chase Cancer Center. Using a panel of MoAbs should help to overcome the differences in sensitivity and specificity among them. Control cell lines, KB-3-1 and KB-8-5 will be used for MDR negative and positive controls. In addition, MDR1 gene expression and clinical outcome will be correlated with cathepsin D, erb B2 oncogene and nm23 expression, other molecular markers implicated in the biological behavior of breast cancer (23,24,25). These will be done in collaboration with Dr. Jose Russo. This analysis is qualitative whereas the RNA assays described below are quantitative.

5.22 Measurement of MDR-1 transcript levels

MDR-1 transcript levels will be measured by three methods including slot blot, RNase protection assay and RT-PCR. Each of these methods has advantages and disadvantages. We have the advantage of comparing these different techniques that vary in sensitivity, specificity and ability to quantify MDR-1 transcripts in a prospective fashion in a well defined group of patients. Such an analysis may help to explain the disparate results of Merkel et al who detected no expression of MDR1 gene expression in breast cancer specimens compared with many of the more recent studies which show a higher percentage of breast cancer samples express MDR1 RNA or protein (8).

Total cellular RNA will be extracted by homogenization in guanidium isothiocyanate followed by acid-phenol extraction (25). KB-3-1 is the drug-sensitive parental KB (HeLa) cell line. KB-8-5, which is four times as resistant to doxorubicin and six times as resistant to vinblastine, will be used as the positive drug resistant control cell line as we demonstrated in previous studies (5,26). The KB-8-5 cell line has increased levels of MDR-1 mRNA without gene amplification as is seen most commonly in clinical specimens (5,27). These cell lines will be used as controls in all of the proposed experiments.

5.221 Slot Blot Analysis

Slot blot analysis has the advantage over Northern blot analysisin that it is semi-quantitative. These assays in our hands are sensitive and reproducible. Serial dilutions of each sample of total RNA will be applied to each well of a Schleicher and Schuell slot blot apparatus and transferred to nitrocellulose filters. The filters will be prehybridized and then hybridized with the MDR-1 cDNA probe 5A (5). Hybridization with a nick-translated actin probe will be performed to control for RNA loading as in previous experiments (5). Probe 5A, which encodes about one-third of the coding region of the full-length MDR1 cDNA, will be labeled by nick translation prior to the use in RNA slot blot analyses as used in our previous studies (5,28).

5.222 RNase Protection Assay

When adequate RNA is available we will perform RNA protection assays. This assay is extremely specific and allows for mapping of the starting sites of MDR1 transcription. An MDR1 genomic fragment of 785 base pairs (bp) will be used to make a riboprobe with SP6 polymerase for the RNase protection assays as we have previously demonstrated (5).

5.223 Reverse Transcription-Polymerization Chain Reaction (RT-PCR)

RT-PCR offers the advantage over the above methods of being extremely sensitive to detect low levels of MDR1 transcripts either when the tumor contains low levels of MDR1 RNA or when only a few cells express the MDR1 gene. Using the competitive template described below will enable us to quantitate the amount of MDR1 RNA present.

Total RNA will be reverse transcribed using random primers (commercially available), and Mo/MEV reverse transcriptase (commercially available)(30). The reverse transcription product or control cDNA will be used as the template in the Polymerization Chain Reaction (PCR) using two MDR1 specific primers, LJG P1 and LJG P2 under the proper PCR conditions (29).

While other mechanisms of adriamycin resistance are not the focus of this proposal, we will have a unique data set of breast tumor RNA to measure glutathione-S-transferase pi (GST pi) and topoisomerase II (30,31,32). Resistance to topoisomerase II inhibitors (i.e. adriamycin and

epipodophyllotoxin) can be due to changes of a number of parameters such as reduced topoisomerase II levels or mutant topoisomerase II. Less cleavable complexes are invariably detected in cells with reduced topoisomerase II levels. Although the best analysis of topoisomerase II is a functional assay of DNA topoisomerase II measuring decatenation, we will not have adequate tissue to accomplish this. Since RNA will have already been extracted we will use published cDNA probes and/or design PCR primers to measure GST pi and topoisomerase II RNA by slot blot analysis and RT-PCR.

5.3 Treatment Administration Schedule

Treatment with Adriamycin

5.31 Treatment

Adriamycin 30 mg/m^2 IV, Day 1,2 and 3

Repeat every 3 weeks X 4

5.32 Calculate dose based on actual body weight.

5.33 Adriamycin (doxorubicin) Administration

Adriamycin should always be administered by side-arm push through a running IV solution to help avoid extravasation.

Emergency anaphylaxis agents, such as epinephrine, diphenhydramine, and glucocortoid for IV should be available in the event of an acute reaction to a first dose of Adriamycin.

5.4 Adverse Reaction Reporting Requirements

ADR REPORTING FOR IND DRUGS - PHASE II & III

5.41 The following adverse reactions must be reported to ECOG and NCI in the manner described below.

Commercial Agent Adriamycin

	Gr 1-5 unexpected[1,2]	Death due to Rx or within 30 days of Rx[3]
ECOG ADR Form to NCI within 10 days	X	X
ECOG ADR Form to ECOG Coordinating Center within 10 days	X	X
ECOG ADR Form to drug sponsor within 10 days	X	

1 Any unexpected toxicity not reported in the literature or the package insert must be reported.
2 Grade 4 expected myelosuppression need not be reported but should be documented on flow sheets.
3 Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.

NCI Telephone Number: (301) 230-2330
NCI FAX Number: (301) 230-0159
NCI Mailing Address:
IDB
P.O. Box 30012
Bethesda, MD 20824

ECOG Telephone Number: (617) 632-3610
ECOG Mailing Address:
ECOG Coordinating Center
Frontier Science
ATTN: ADR
303 Boylston Street
Brookline, MA 02146-7648

ECOG requires ADRs to be reported on the Adverse Reaction (ADR) Form for Investigational Drugs (#391RF). The form must be signed by the treating investigator.

5.43 Non-Treatment Related Toxicities

If a toxicity is felt to be outside the definitions listed above and unrelated to the protocol treatment, this must be clearly documented on the ECOG Flow Sheets which are submitted to the ECOG Coordinating Center (ATTN: DATA) according to the Records to be Kept Section (11.0). This does not in any way obviate the need for reporting the toxicities described above.

5.5 Dose Modifications

There will be no dose escalations.

All toxicities should be graded according to the Common Toxicity Criteria (Appendix II).

5.51 Hematologic Toxicity

5.511 Day 1 of each course:

Granulocytes		Platelets	Dose Adriamycin
$\geq 500/mm^3$	and	$\geq 10^5/mm^3$	100%

$<1500/mm^3$	or	$<10^5/mm^3$	Hold therapy until granulocytes ≥ 1500 and platelets $\geq 10^5$

5.512 Any patient experiencing any of the following will have Adriamycin reduced by 25%:

5.5121 Febrile neutropenic episode ($\geq$ 38.5°C) between courses
5.5122 An absolute granulocyte count <500/ul for 15 days
5.5123 Bleeding episode with a platelet count $\leq$ 40,000/mm^3
5.5124 Platelet count $\leq$ 20,000/mm^3 with or without a bleeding episode
5.5125 Failure to recover counts for retreatment on day 22

If, on a subsequent course, these toxicities recur, adriamycin will be reduced by another 25%.

Growth factor support may be used at the discretion of the treating physician.

5.52 Gastrointestinal Toxicity

5.521 Nausea and/or vomiting should be controlled with standard antiemetics.

5.522 If mucositis is present on day 1 of any cycle, the treatment should be withheld until the mucositis has cleared. If acute Grade 3 or 4 mucositis occurs, Adriamycin should be given at 75% when the mucositis is completely cleared for all subsequent courses.

5.523 Grade 3 or 4 diarrhea is sufficient reason to reduce the Adriamycin to 75% (after resolution of diarrhea) for all subsequent courses. For grade 2, the dose should be held until the diarrhea clears, and resumed at full dosage.

5.53 Hepatic Toxicity

SGOT		Bilirubin (mg/dl)	% Dose to Give Adriamycin
<2 X baseline	and	$\leq$ 1.3 X baseline	100%
$\geq$2-5 X baseline	and/or	1.4 - 2.0 X baseline	50%
>5 X baseline	and/or	2.1 - 3.3 X baseline	25%
>5 X baseline	and/or	>3.3 X baseline	10%

Note: Patients with >5 X baseline SGOT or >3.3 x baseline bilirubin should be evaluated for possible progressive hepatic disease.

5.54 Anaphylaxis (See Section 5.111 for preventive measures)

5.541 Any patient who develops major hypersensitivity events (hypotension or dyspnea), despite Section 5.111 guidelines, should be removed from the study.

5.542 Skin rash has been reported. If transient, and not accompanied by other allergic symptoms, the patient may continue on study.

5.55 Cardiovascular

Patients who develop chest pain, hypotension, or arrhythmia will require careful evaluation to be certain there has been no significant myocardial damage. If such events occur, drug infusion (of Adriamycin) should be discontinued. When the chest

pain or hypotension resolves, the infusion may be resumed at 50% of the previous rate. When the arrhythmia resolves, infusion may be resumed at 100%. If the patient develops signs of congestive heart failure or reduction of left ventricular ejection fraction (LVEF) > 15%, or reduction to a LVEF < 50%, doxorubicin should be discontinued.

5.56 Other Toxicities

For any Grade 4 toxicity not mentioned above, the treatment should be withheld until patient recovers completely or Grade 1 toxicity, then the treatment should be resumed at 50% dose. After dose reduction, if the patient tolerates treatment well (i.e. only Grade 1 toxicity occurs) then the Adriamycin should be increased by 25% with the goal to return to the 100% dose level. Reversible grade 3 toxicities should result in a 25% reduction in Adriamycin. If no grade 3 toxicity occurs on the subsequent cycle, the doses may be increased to 100%. For Grade 1 or 2 toxicities no dose reduction should be made.

All toxicities should be graded according to the Common Toxicity Criteria (see Appendix II).

5.6 Supportive Care

5.61 All supportive measures consistent with optimal patient care will be given throughout the study.

5.7 Duration of Therapy

5.71 Patients will be treated with 4 cycles of doxorubicin unless there is evidence of progressive disease or patients develop overwhelming toxicity prohibiting continuation of therapy.

5.72 If patients progress while on therapy, a biopsy of the breast tumor should be obtained for MDR expression analysis as described in section 5.1.

6.0 MEASUREMENT OF EFFECT

SOLID TUMOR RESPONSE CRITERIA

6.1 ECOG Solid Tumor Response Criteria

6.11 Methods of Malignant Disease Evaluation

6.111 Measurable, Bidimensional

Malignant disease measurable (metric system) in two dimensions by ruler or calipers with surface area determined by multiplying the longest diameter by the greatest perpendicular diameter (i.e., metastatic pulmonary nodules, lymph nodes, and subcutaneous masses). Malignant disease with sharply defined borders visualized by ultrasonography or computerized axial tomography is considered measurable. Repeat studies should be performed at the same pretherapy site(s) of malignant disease.

6.112 Measurable, Unidimensional

Malignant disease measurable (metric system) in one dimension by ruler or calipers (i.e., mediastinal adenopathy, malignant hepatomegaly, or abdominal masses).

6.113 Nonmeasurable, Evaluable

Malignant disease evident on clinical (physical or radiographic) examination, but not measurable by ruler or calipers (i.e., pelvic and abdominal masses, lymphangitic or confluent multinodular lung metastases, skin metastases, ascites or pleural effusions known to be caused by peritoneal or pleural metastases and uninfluenced by diuretics, liver scans, bone scans, gallium scans, deviated or obstructed ureters or gastrointestinal tract, and masses with poorly defined borders on ultrasonography or computerized axial tomography).

6.1131 Photographs should be taken prior to and during therapy to document response.

6.1132 CT scans can be used to evaluate response.

6.1133 Chemical parameters and biologic markers will be measured during therapy, but will not be used to evaluate response, unless specifically stipulated in individual protocols. Normalization of hyperbilirubinemia known to be caused by malignant disease may be used as an evaluable response if specified in individual protocols.

6.2 Definitions of Response by Organ Site Involvement

6.21 Complete Response

6.211 Clinical

Complete disappearance of all clinically detectable malignant disease for at least 4 weeks. a patient who has radiographic evidence of bony metastases prior to therapy has to have normalization of radiographs or complete sclerotic healing of lytic metastases in association with a normal bone scan. a patient with an abnormal bone scan and normal radiographs prior to therapy has to have normalization of the bone scan.

6.212 Pathologic

Pathologic proof of a clinically complete response after rebiopsying areas of known malignant disease.

6.22 Partial Response

Greater than or equal to 50% decrease in tumor size for at least 4 weeks without increase in size of any area of known malignant disease of greater than 25%, or appearance of new areas of malignant disease.

6.221 Measurable, Bidimensional

Greater than or equal to a 50% decrease in tumor area (multiplication of longest diameter by the greatest perpendicular diameter), or a 50% decrease in the sum of the products of the perpendicular diameters of multiple lesions in the same organ site for at least 4 weeks.

6.223 Nonmeasurable, Evaluable

Definite improvement in evaluable malignant disease estimated to be in excess of 50% and **agreed upon by 2 independent investigators.**

6.2231 Serial evaluations of chest x-rays (i.e., confluent multinodular and lymphangitic metastases, malignant pleural effusions) and physical measurements (i.e., abdominal girth) should be documented in the records and by photograph when practical.

6.2232 The response should last for at least 4 weeks.

6.23 Stable

No significant change in measurable or evaluable disease for at least 4 weeks (greater than or equal to 12 weeks for bony metastases).

6.231 No increase in size of any known malignant disease.

6.232 No appearance of **new** areas of malignant disease.

6.233 This designation includes decrease in malignant disease of less than 50%, or decrease in unidimensional measurable disease of less than 30%, or increase in malignant disease of less than 25% in any site.

6.234 No deterioration in ECOG performance status of greater than or equal to 1 level related to malignant disease.

6.24 Progression

Significant increase in size of lesions present at the start of therapy or after a response, or appearance of new metastatic lesions **known not** to be present at the start of therapy or stable objective disease associated with a deterioration in ECOG performance status of greater than or equal to 1 level related to malignancy.

6.241 Measurable, Bidimensional, and Unidimensional

6.2411 Greater than or equal to 25% increase in the area of any malignant lesions greater than 2 cm^2 or in the sum of the products of the individual lesions in a given organ site (comparison of products of the longest diameter by the greatest perpendicular diameter).

6.2412 Greater than or equal to 50% increase in the size of the product of diameters if only one lesion is available for measurement and was less than or equal to 2 cm^2 in size at the initiation of therapy.

6.2413 Greater than or equal to 25% increase in the sum of the liver measurements below the costal margins and xyphoid.

6.2414 Appearance of **new** malignant lesions.

6.242 Nonmeasurable, Evaluable

6.2421 Definite increase in the area of malignant lesions estimated to be greater than 25%.

6.2422 Appearance of **new** malignant lesions.

6.2423 Increase in size or number of bony metastases (pathologic fractures do not represent progression unless there is a documented increase in bony disease).

6.243 Nonmeasurable, Nonevaluable

Definite evidence of **new** clinically detectable (physical or radiographic) malignant disease.

6.25 No Evidence of Disease (NED)

Lack of clinically identifiable malignant disease in nonmeasurable, nonevaluable or adjuvant patients.

6.3 Evaluation of Patient's Total Response

6.31 Organ Site Evaluation

6.311 Record responses as complete (CR), partial (PR), stable (S), progression (P) or NED under appropriate methods of evaluation.

6.312 If more than one type of evaluation method exists for a given organ site, each must be recorded separately.

6.313 If there is more than one measurable lesion per organ site, an organ site PR occurs if there is a greater than 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions.

6.314 In patients with measurable disease, the worst response will prevail in determining response by organ site.

6.315 Stabilization of evaluable disease will not detract from a PR of measurable disease by organ site, but will reduce a CR to a PR.

6.316 Progression in any classification of measurability or evaluability in an organ site shall prevail as the response for that organ site.

6.32 Objective Total Patient Response

6.321 Progression occurs if any previously measurable or evaluable malignant lesions fulfill progression criteria or new malignant lesions **not known** to be present at the start of therapy develop.

6.322 Organ site stabilizations will not detract from a total patient PR in the presence of other organ site PR's and CR's.

6.323 Stabilization of evaluable disease does not detract from CR's or PR's in measurable sites, but the patient's overall response should be a PR.

6.324 Patients with a deterioration in ECOG performance status of greater than or equal to 1 level related to malignant disease are considered progressors.

6.33 Onset of Response

The time between initiation of therapy and the onset of PR or CR.

6.34 Duration of Response

Time from onset of PR or CR , whichever occurs first,(even if patient later has a CR) until objective evidence of progression.

6.35 Subjective Patient Response

In order to evaluate the quality of life during therapy, the investigator must summarize the changes in performance status and evaluate whether these changes are due to malignant disease, treatment or to unrelated factors.

7.0 STUDY PARAMETERS

Advanced Disease Protocols

a. All scans and x-rays should be done ≤ 6 weeks before randomization/registration.

b. Scans or x-rays used to document measurable or evaluable disease should be done within 2 weeks prior to randomization/registration.

c. CBC with differential, LFT's should be done ≤ 2 weeks before randomization/registration.

d. All chemistries should be done ≤ 2 weeks before randomization/registration - unless specifically required on Day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to randomization/registration.

e. Hgb, Hct, WBC, Plt should be done ≤ 2 weeks before randomization/registration but if abnormal, they must be repeated < 48 hours prior to randomization/registration.

NOTE: When filling out these pre study results on the ECOG flow sheets, please make sure that ALL relevant dates are clearly given. Do NOT put all the results under the date for Day 1 of protocol treatment unless they were actually done that day. Record the actual dates.

For follow up Hgb, Hct, WBC, Plt, these tests should be done within 48 hours of the day of treatment.

	Pre-Treatment	Every Cycle	At end of 4 Cycles or Progressive Disease	Off Treatment Every 4 Months
Physical Examination	X	X	X	X
Tumor Measurements	X	X[7]	X	X
Performance Status	X	X	X	X
Height & weight	X	X	X	X
WBC (diff), Hgb, Plt[1]	X	X[1]	X	X
Chest X-ray	X			X[2]
Serum Creatinine	X	X	X	X
SGOT & Bilirubin	X	X	X	X
Urinalysis	X			
CT Chest	X		X[5]	
Bone Scan	X		X[5]	
Xrays of Bone Scan Lesions or Skeletal Survey	X[6]		X[5]	
MUGA Scan or Echocardiogram	X[3]		X[3]	
EKG	X		X[4]	
MRI Bone Marrow; MRS	X		X[5]	
Tumor biopsy for MDR analysis	X		X	

1 To be done on Days 8,15 and 21 (or day 1 prior to retreatment) of each cycle for all patients.
2 Annually unless symptomatic or new lesions.
3 Obtained prior to therapy, at crossover and at completion of study. Report on flow sheets.
4 If clinically indicated.
5 If lesions on previous examination.
6 Required only if bone scan is abnormal or known lesions are present.
7 All areas of measurable disease will be measured each cycle unless measurements require a CT scan. If the patient has only one site of measurable disease that is documented only by CT scan, then CT scans will be required after 4 cycles. If the patient has multiple sites of measurable disease including at least one site that is not measured by CT, then the sites not requiring CT imaging will be measured each cycle and sites requiring CT imaging will be imaged by CT at the end of 4 cycles.

8.0 DRUG FORMULATION AND PROCUREMENT

8.1 Doxorubicin

8.11 Other Names

Adriamycin, Rubex, Adriamycin RDF, Adriamycin PFS, hydroxydaunorubicin, hydroxydaunomycin, ADR

8.12 Classification

Anthracycline antibiotic.

8.13 Mode of Action

Intercalation between adjoining nucleotide pairs in the DNA helix causes inhibition of DNA and DNA-dependent RNA synthesis. Free radical generation is responsible for cardiac toxicity. Doxorubicin also inhibits topoisomerase II.

8.14 Storage and Stability

Rubex or Adriamycin RDF intact vials are stable protected from light at room temperature. Adriamycin PFS vials must be refrigerated. Reconstituted solutions are stable for 24 hours at room temperature and 48 hours under refrigeration. The Adriamycin RDF 150 mg multidose vial is stable after reconstitution for 7 days at room temperature or 15 days if refrigerated and protected from sunlight.

8.15 Preparation

Add 5, 10, 25, 50, or 75 ml of preservative-free normal saline to the 10, 20, 50, 100, or 150 mg vial to produce a solution containing 2 mg/ml.

8.16 Route of Administration

Side-arm push through a running IV solution.

8.17 Incompatibilities

Physically incompatible with heparin, fluorouracil, aminophylline, cephalothin, dexamethasone, diazepam, hydrocortisone, and furosemide.

8.18 Compatibilities

Stable with vincristine in normal saline for five days at room temperature protected from light. Also compatible in solution with cyclophosphamide.

8.19 Availability

Commercially available as powder for injection in 10, 20, 50, 100, 150 mg vials, and as 2 mg/ml solution for injection in 10, 20, 50, and 200 mg vials.

8.110 Side Effects

8.1101 Hematologic: Leukopenia (dose-limiting), also thrombocytopenia and anemia. Nadir 10-14 days, recovery in 21 days.

8.1102 Dermatologic: Alopecia, usually complete; hyperpigmentation of nailbeds and dermal creases; radiation recall.

8.1103 Gastrointestinal: Nausea and vomiting, sometimes severe; anorexia, diarrhea; mucositis, especially with daily x 3 schedule.

8.1104 Cardiovascular: Arrhythmias, ECG changes; rare sudden death. Congestive heart failure due to cardiomyopathy related to total cumulative dose; risk is greater with total doses >550 mg/m^2, mediastinal irradiation pre-existing cardiac disease, advanced age; risk is reduced with weekly or continuous infusion regimens.

8.1105 Other: Red discoloration of urine; fever; anaphylactoid reaction may enhance cyclophosphamide cystitis or mercaptopurine hepatotoxicity.

8.1106 Local effects: Vesicant if extravasated; flush along vein, facial flush.

8.111 Nursing Implications

8.1111 Monitor CBC, platelet counts.

8.1112 Vesicant - do not extravasate. Refer to extravasation protocol if inadvertent infiltration occurs.

8.1113 Advise patient of alopecia. Instruct on how to obtain wig, hairpiece, etc. Hair loss generally occurs 2-4 weeks after injection and is usually complete.

8.1114 Advise patient of red discoloration of urine for 24 hours after administration of the drug.

8.1115 Administer antiemetics as indicated.

8.1116 Assess for stomatitis and treat symptomatically. Generally occurs 7-10 days after injection.

8.1117 Be aware of "Adria" flare - most common reaction consists of an erythematous streak up the vein. It is associated with urticaria and pruritus. Occasionally the use of corticosteroids and/or antihistamines has been useful.

8.1118 Monitor for signs and symptoms of cardiomyopathy. Calculate total cumulative dose with each administration.

9.0 STATISTICAL CONSIDERATIONS

The primary goal of this study is to assess MDR1 gene expression in patients with locally advanced or inflammatory breast cancer at diagnosis and following single agent induction therapy. These assessments will be examined to estimate both the proportion of patients with MDR1 gene expression de novo, and the proportion without de novo expression who do express this gene following single agent induction therapy.

We seek to enroll 30 patients on this study, with the expectation that at least 25 patients will provide fully evaluable sequential samples for the MDR1 assays and will be assessable for response to induction therapy. Data on MDR1 gene expression and response to single agent induction therapy collected on this study will be combined with data collected on other ECOG studies (E4193, EXXXX) to explore the association between MDR1 and resistance to doxorubicin therapy. If a total of 30 patients are fully evaluable for response to induction therapy and for MDR1 gene expression at the completion of that therapy, we will have 81% power to detect the difference between a response rate of 80% in patients without MDR1 expression and a response rate of 20 % in patients with MDR1 expression, assuming that 20% of patients will have MDR1 expression and testing at the 10% two-sided significance level. If 50% of patients express MDR1, we will still be able to detect, with 80% power, the difference between a 75% response rate and a 25% response rate, testing at the two-sided 10% significance level.

We will also examine the concordance of the various methods of measuring MDR1 gene expression in all patients enrolled in this study, and in the patients will fully analyzable sequential samples.

10.0 PATHOLOGY REVIEW

10.1 The clinical investigator and the submitting pathologist have the responsibility for submitting representative diagnostic material for review and classification. When a patient is placed on study, the submitting pathologist and data manager should refer to Appendix III (Pathology Submission Guidelines) which provides the following:

10.11 Instruction Sheet from ECOG Pathology Coordinating Office providing details for the Submission of Pathology Materials.

10.12 Memorandum from Barbara Wolf, M.D., Executive Director, ECOG PCO, providing details for the Submission of Pathology Materials.

10.13 a list of required materials.

10.14 An ECOG Pathology Material Submission Form (#050).

10.2 The materials required for this protocol are:

10.21 ECOG Pathology Material Submission Form (#050), Parts a & B completed.

10.22 a copy of the surgical pathology report. In addition to the surgical pathology report, if immunologic studies have been performed at the home institution, it is necessary that these be forwarded as well. Please identify the clinical status of the submitted material (i.e., pretreatment as opposed to remission and relapse).

10.23 LIST BLOCKS AND/OR SLIDES REQUIRED AT THE TIME OF REGISTRATION TO STUDY

Note Submission of pathologic materials is mandatory in order for the patient to be considered evaluable. Failure to submit pathologic materials will render the case ineligible.

Note a copy of the completed submission form will be sent to the Coordinating Center by the Pathology Coordinating Office.

10.24 When insufficient material is available, at least one (1) Wright stained smear must be forwarded for the patient to be eligible.

10.3 LIST BLOCKS AND/OR SLIDES REQUIRED AT REMISSION AND/OR RELAPSE

10.4 Routing

The required materials must be submitted within 1 month of patient registration to:

ECOG Coordinating Center
Frontier Science
Attn: Pathology
303 Boylston Street
Brookline, MA 02146-7648

11.0 RECORDS TO BE KEPT

The following forms must be submitted to the ECOG Coordinating Center, 303 Boylston Street, Brookline, MA 02146 (ATTN: DATA).

Form	To Be Submitted
* _____ On-Study Form	Within one week of registration

ECOG CTC Flow Sheet	Every (month/3 months) while on treatment
ECOG Measurement Form ECOG Follow-Up Form	Every (month/3 months) while on study treatment and at completion of treatment
Parts a, B, C, D, E * Parts a, B	Off Treatment: • every 3 months if patient is <2 years from study entry • every 6 months if patient is 2-5 years from study entry • every 12 months if patient is > 5 years from study entry
Adverse Reaction (ADR) Form for Investigational Drugs	Within 10 days of reportable event as defined in Section _____

* These forms are to be submitted for all canceled patients according to the above schedule.

12.0 PATIENT CONSENT AND PEER JUDGMENT

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

13.0 REFERENCES

1. Sorace RA, Lippman ME. Locally advanced breast cancer. In Lippman ME, Lichter a, and Danforth DN, editors. Diagnosis and management of breast cancer, Philadelphia, 1988, WB Saunders, pp 272-295.

2. Haagensen CD, Stout AP. Carcinoma of the breast. II. Criteria of operability. Ann Surg 118:859, 1943.

3. Bryant T. Disease of the breast. Wood's Medical Surg Monograph 4:35, 1989.

4. Haagensen, CD. Inflammatory Carcinoma. In Haagensen, CD, editor. Diseases of the breast, ed 2, Philadelphia, 1971, WB Saunders, pp 566-584.

5. Lee BJ , Tannenbaum, NE. Inflammatory carcinoma of the breast: a review of twenty-eight cases from the breast clinic of the Memorial Hospital. Surg Gynecol Obstet 39:580, 1924.

6. Burton GV, Cost EB, Leight, GS, et al. Inflammatory breast carcinoma. Effective multimodal approach. Arch Surg 122:1329, 1987.

7. Fastenberg NA, Buzdar, AU, Montague, ED, et al. Management of inflammatory carcinoma of the breast. A combined modality approach. AM J Clin Oncol 8:134, 1985.

8. Nussbaum H, Kagan AR, Gilbert H, et al. Management of inflammatory breast carcinoma. Breast Disease of the Breast 1:25, 1977.

9. Schafer P, Alberto P, Forni M, et al. Surgery as part of a combined modality approach for inflammatory breast cancer. Cancer 59:1063, 1987.

10. Swain, SM , Lippman ME. Treatment of patients with inflammatory breast cancer. Important Adv Oncol 129:1989.

11. Balawajder I, Antich PP, Boland J. An analysis of the role of radiotherapy alone and in combination with chemotherapy and surgery in the management of advanced breast cancer. Cancer 51:574, 1983.

12. Barker JL, Nelson AJ, Montague ED. Inflammatory carcinoma of the breast. Radiology 121:173, 1976.

13. Bedwinek J, Rao DV, Perez C, et al. Stage III and localized stage IV breast cancer: Irradiation alone vs. irradiation plus surgery. Int J Radiat Oncol Biol Phys 8:31, 1982.

14. Fields JN, Perez CA, Kuske RR, et al. Inflammatory carcinoma of the breast: Treatment results in 107 patients. Int J Radiat Oncol Biol Phys 17:249, 1989.

15. Fletcher GH. Clinical dose-response curves of human malignant epithelial tumors. Br J Radiol 46:1, 1973.

16. Rouesse J, Friedman S, Sarrazin D, et al. Primary chemotherapy in the treatment of inflammatory breast carcinoma: A study of 230 cases for the Institute Gustave-Roussy. J Clin Oncol 4:1765, 1986.

17. Delena M, Varini M, Zucali R, et al. Multinodal treatment for locally advanced breast cancer. Cancer Clin Trials 4:229, 1981.

18. Hortobagyi GN, Ames FC, Buzdar AU, et al. Management of Stage III primary breast cancer with primary chemotherapy, surgery and radiation therapy. Cancer 62:2507, 1988.

19. Hortobagyi G, Holmes F, Frye D, et al. Comparison of two adjuvant chemotherapy programs for locally advanced breast cancer. Am Soc Clin Oncol 9:21, 1990 [abstract].

20. Jacquillat C, Baillet F, Weil M, et al. Results of a conservative treatment combining induction (neoadjuvant) and consolidation chemotherapy, hormonotherapy, and external and interstitial irradiation in 98 patients with locally advanced breast cancer (IIIA-IIIB). Cancer 61:1977, 1988.

21. Perloff M, Lesnick GJ, Korzum A, et al. Combination chemotherapy with mastectomy or radiation for stage III breast carcinoma. A Cancer and Leukemia Group B study. J Clin Oncol 6:261, 1988.

22. Cardenas J, Ramirez T, Noriega J, et al. Multidisciplinary Therapy for locally advanced Breast Cancer (LABC). An Update Proc Am Soc Clin Oncol 6:4261, 1987.

23. Jones RB, Holland JF, Bhardwaj S, et al. A Phase I-II study of Intensive-Dose Adriamycin for advanced Breast Cancer. J Clin Oncol 5:172-177, 1987.

24. Ragaz J, Knowling M, Jones G, et al. Comparability of preoperative (neoadjuvant) (Pre) and Postoperative (post) Therapy for Locally advanced breast cancer (LABC). Proc Annu Meet Am Assoc Cancer Res 28:197, 1987 [abstract].

25. Kantarjian HM, et al. The management of locally advanced breast cancer: A combined modality approach. Eur J Cancer Clin Oncol 20 (11); 1353-1361, 1984.

26. Swain SM, Sorace RA, Bagley CS, et al. Neoadjuvant chemotherapy in the combined modality approach of locally advanced non-metastatic breast cancer. Cancer Res 47:14 3889-94, 1987.

27. Thomas WW Jr., McNeese MD, Fletcher GH, et al. Multimodal treatment for inflammatory breast cancer. Int J Radiat Oncol Biol Phys 17:739, 1989.

28. Zylberberg B, Salat-Baroux J, Ravine JR, et al. Initial chemoimmunotherapy in inflammatory carcinoma of the breast. Cancer 49:1537, 1982.

29. Hagelberg RS, Jolly PC, Anderson RP. Role of surgery in the treatment of inflammatory breast carcinoma. Am J Surg 148:125, 1984.

30. Israel L, Breau JL, Morere JF. Two years of high-dose cyclophosphamide and 5-Fluorouracil followed by surgery after 3 months for acute inflammatory breast carcinoma. A phase II study of 25 cases with a median follow-up of 35 months. Cancer 57:24, 1986.

31. Beidler JL, Riehn H. Cellular resistance to actinomycin D in Chinese hamster ovary cell in vitro: cross resistance, radiographic and cytogenetic studies. Cancer Res 30:1174-1184, 1970.

32. Croop JM, Gros P, Housman DE. Genetics of multidrug resistance. J Clin Invest 81:1303-1309, 1988.

33. Endicott J, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. Ann Rev Biochem 58:137-171, 1989.

34. Kane SE, Pastan I, Gottesman MM. Genetic basis of multidrug resistance of tumor cells. Bioenergetics and Biomembranes 22:593-617, 1990.

35. Goldstein LJ, Galski H, Fojo A, Willingham MC, Lai S, Gazdar A, Pirkir R, Green A, Crist W, Brodeur G, Lieber M, Cossman J, Gottesman MM, Pastan I. Expression of a multidrug resistance gene in human cancer. J Natl Cancer Inst 81:116-124, 1989.

36. Wallner J, Depisch D, Hopfner M, Haider K, Spona J, Ludwig H, Pirker R. MDR1 gene expression and prognostic factors in primary breast carcinomas. Eur J Cancer 27, 1352-1355, 1991.

37. Keith WN, kStallard S, Brown R. Expression of mdr1 and gst II in human breast tumors: comparison to in vitro chemosensitivity. Br J Cancer 61:7122-716, 1990.

38. Merkel DE, Fugua SAW, Tandona AK, Hill SM, Buzdar AV, McGuire WL. Electrophoretic analysis of 248 clinical breast specimens for p-GP overexpression or gene amplification. J Clin Oncol 7:1129-1136, 1989.

39. Schneider J, Bak M, Efferth TH, Kaufman M, Mattern J, Volm M. P-glycoprotein expression in treated and untreated human breast cancer. British J Cancer 60:815-818, 1989.

40. Wishart GC, Plumb JA, Going JJ, McNicol AM, McArdle CS, Tsuruo T, Kaye SB. P-glycoprotein expression in primary breast cancer detected by immunocytochemistry with two monoclonal antibodies. Br J Cancer 62:758-761, 1990.

41. Ro J, Sahin A, Ro JY, Fritsche J, Hortobagyi G, Blick M. Immunohistochemical analysis of P-glycoprotein expression correlated with chemotherapy resistance in locally advanced breast cancer. Hum Pathol 21:787-791, 1990.

42. Verrelle P, Meissonnier F, Fonck Y, Feillel V, Dionet C, Kwiatkowski F, Plagne R, Chassagne J. Clinical relevance of immunohistochemical detection of multidrug resistance P-glycoprotein in breast carcinoma. J Natl Cancer Inst 83:111-116, 1991.

43. Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S. Tissue distribution of P-glycoprotein encoded by a multidrug resistant gene as revealed by a monoclonal antibody, MRK16. Cancer Res 48:4611-4614, 1988.

44. Gerlach JH, Bell R, Karakousis C, Slocum HK, Kartner N, Rustum YM, Ling V, Baker RM. P-glycoprotein in human sarcoma: evidence for multidrug resistance. J Clin Oncol 5:1452-1460, 1987.

45. Sanfilippo O, Ronchi E, DeMarco C, DiFronzo G, Silvestrini R. Expression of P-glycoprotein in Breast Cancer Tissue and in vitro Resistance to Doxorubicin and Vincristine. Eur J Cancer 7, 155-158, 1991.

46. Wishart GC, Plumb JA, George WD, Kaye SB. P-glycoprotein expression is found in stromal cells of

breast cancer but not those of normal breast. Br J Cancer 64 (Suppl 15):8, 1991.

47. Bronchud MH, Margison JM, Howell AH, et al. Comparative Pharacokinetics of Escalating Doses of Doxorubicin in Patients with Metastatic Breast Cancer. Cancer Chemother Pharmacol 25: 435-439, 1990.

48. Brambilla C, Rossi A, Bonfante V, et al. Phase II Study of Doxorubicin versus Epirubicin in Advanced Breast Cancer. Cancer Treat Rep 70:261-266, 1986.

49. Bronchud MH, Howell A, Crowther D, et al. The Use of Granulocyte Colony-Stimulating Factor to Increase the Intensity of Treatment with Doxorubicin in Patients with Advanced Breast and Ovarian Cancer. Br J Cancer 60: 121-125, 1989.

50. Carmo-Pereira J, Costa FO, Henriques E, et al. A Comparison of Two Doses of Adriamycin in the Primary Chemotherapy of Disseminated Breast Carcinoma. Br J Cancer 56: 471-473, 1987.

51. Steiner R, Stewart JF, Cantwell BJM, et al. Adriamycin Alone or Combined with Vincristine in the Treatment of Advanced Breast Cancer. Eur J Cancer Clin Oncol 19: 1553-1559, 1983.

Induction with Adriamycin in
Inoperable Locally Advanced and Inflammatory Breast Cancer to
Evaluate for Multidrug Resistance
A Registration Study

APPENDIX I

Suggested Patient Consent Form

Research Study

I, _____, willingly agree to participate in this study which has been explained to me by Dr. _____. This research study is being conducted by the Eastern Cooperative Oncology Group and by _____.
(Institution)

Purpose of the Study

It has been explained to you that you have breast cancer which is not controllable with surgery or radiation therapy alone and your physician has decided that chemotherapy is the best first treatment option available. You have been invited to participate in this research study, which involves the use of the drug adriamycin, which has been previously tested in your stage disease. The purpose of this study is to: 1) slow or stop the growth of my tumor; 2) gain information about your disease; 3) help identify better treatments for cancer of the breast.

DESCRIPTION OF PROCEDURES

This study involves treatment with adriamycin.

After you have been registered to this study, you will receive adriamycin through your vein every 3 weeks for a total of four treatments. Your physician and you will then decide what the next best treatment is for you depending on how your breast cancer responds to adriamycin

This study also involves a biopsy of your breast cancer before and after treatment with Adriamycin so that your doctors can learn how Adriamycin works. If you have a lumpectomy or a mastectomy after Adriamycin treatment, a sample of your tumor will be used so that you will not require another biopsy. Pieces of tumor tissue will be sent to a special laboratory and studied prior to starting treatment and at the time that you finish or stop therapy. The results of these studies will not be used to change your therapy.

RISKS AND DISCOMFORTS

Chemotherapy drugs often have side effects. The drug used in this program may cause all, some, or none of the side effects listed. In addition, there is always the risk of very uncommon or previously unknown side effects occurring.

Adriamycin may cause mouth sores, fever, chills, nausea/vomiting, hair loss, changes in skin and nail texture and/or color, skin rash, abnormal amount of uric acid in the blood, vein inflammation, lowering of blood cell counts and platelet counts which can result in bleeding and infection. Rarely, with doses given over a long period of time, Adriamycin may cause irreversible weakening of the heart muscle which may be life-threatening. Adriamycin may damage the skin if it leaks into the tissues surrounding the injection site; occasionally this may result in the need for a skin graft.
Your physician will be checking you closely to see if any of these side effects are occurring. Routine blood and urine tests will be done to monitor the effects of treatment. Many side effects disappear after the treatment is stopped. In the meantime, your doctor may prescribe medication to keep these side effects under control. Other side effects may be long-lasting or permanent. You understand that treatment to help control side effects

could result in added costs. This institution is not financially responsible for treatments of side effects caused by the study drugs.

Risks associated with biopsies include bleeding, infection and allergic reactions to the local anesthetic. In addition, there is always the risk of very uncommon or previously unknown side effects occurring.

Contact Persons

In the event that physical injury occurs as a result of this research, facilities for treatment of injury will be available; however, you will not automatically be provided with reimbursement for medical care or other compensation. For more information concerning the research and research-related risks or injuries, you can notify Dr. _____, the investigator in charge at _____ (Telephone). In addition, you may contact _____ at _____ (Telephone) for information regarding patients' rights in research studies.

Benefits

It is not possible to predict whether or not any personal benefit will result. Possible benefits are remission of tumor and prolonged survival. You have been told that, should your disease become worse, should side effects become very severe, should new scientific developments occur that indicate the treatment is not in your best interest, or should your physician feel that this treatment is no longer in your best interest, the treatment would be stopped. Further treatment would be discussed.

Alternatives

Alternative treatments which could be considered in your case include treatment with other chemotherapies. My doctor can provide detailed information about my disease and the benefits of the various treatments available. You have been told that you should feel free to discuss your disease and your prognosis with the doctor.

The physician involved in your care would be available to answer any questions you have concerning this program. In addition, you are free to ask your physician any questions concerning this program that you wish in the future. You will be advised of the procedures related solely to research which would not otherwise be necessary. These will be explained to you by your physician. Some of these procedures may result in added costs and some of these costs may not be covered by insurance. Your doctor will discuss these with you.

Voluntary Participation

Participation in this study is voluntary. No compensation for participation will be given. You are free to withdraw your consent to participate in this treatment program at any time without prejudice to my subsequent care. Refusing to participate will involve no penalty or loss of benefits. You are free to seek care from a physician of your choice at any time. If you do not take part in or withdraw from the study, you will continue to receive care. In the event that you withdraw from the study, you will continue to be followed and clinical data will continue to be collected from your medical records.

Confidentiality

A record of my progress while on the study will be kept in a confidential form at _____ and also in a computer file at the statistical headquarters of the
(Institution)
Eastern Cooperative Oncology Group. The confidentiality of the central computer record is carefully guarded. During their required reviews, representatives of the Food and Drug Administration (FDA) and the National Cancer Institute (NCI) ___*(and sponsoring agencies, if applicable)*___ may have access to medical records which contain your identity. However, no information by which you can be identified will be released or published. Histopathologic material, including slides, may be sent to a central office for review.

****

I have read all of the above, asked questions, received answers concerning areas I did not understand and I willingly give my consent to participate in this program. Upon signing this form, I will receive a copy.

_____	_____
(Patient Signature)	(Date)
_____	_____
(Witness Signature)	(Date)
_____	_____
(Physician Signature)	(Date)

Induction with Adriamycin in
Inoperable Locally Advanced and Inflammatory Breast cancer to
Evaluate for Multidrug Resistance
A Registration Study

APPENDIX II

COMMON TOXICITY CRITERIA

		0	1	2	3	4
Leukopenia	WBC x 10^3 Granulocytes/Bands Lymphocytes	≥4.0 ≥2.0 ≥2.0	3.0 - 3.9 1.5 - 1.9 1.5 - 1.9	2.0 - 2.9 1.0 - 1.4 1.0 - 1.4	1.0 - 1.9 0.5 - 0.9 0.5 - 0.9	<1.0 <0.5 <0.5
Thrombocyto-penia	Plt x 10^3	WNL	75.0 - normal	50.0 - 74.9	25.0 - 49.9	<25.0
Anemia	Hgb	WNL	10.0 - normal	8.0 - 10.0	6.5 - 7.9	<6.5
Hemorrhage (Clinical)	- - - - -	none	mild, no transfusion	gross, 1-2 units transfusion/episode	gross, 3-4 units transfusion/episode	massive, >4 units transfusion/episode
*Infection	- - - - -	none	mild, no active Rx	Moderate, localized infection requires active Rx	severe, systemic infection requires active Rx, specify site	life-threatening, sepsis, specify site
Fever in absence of infection	- - - - -	none	37.1° - 38.0° C 98.7 °- 100.4° F	38.1 ° - 40.0°C 100.5 ° - 104.0° F	>40.0° C (>104.0° F) for less than 24 hours	>40.0° C (104.0° F) for >24 hrs or fever with hypotension
	• **Fever felt to be caused by drug allergy should be coded as allergy.** • **Fever due to infection is coded under infection only.**					
GU	Creatinine	WNL	< 1.5 x N	1.5 - 3.0 x N	3.1 - 6.0 x N	>6.0 x N
	Proteinuria	No change	1+ or <0.3g% or <3g/l	2-3+ or 0.3 - 1.0g% or 3 - 10g/l	4+ or >1.0g% or >10g/l	nephrotic syndrome
	Hematuria	neg	micro only	gross, no clots	gross + clots	requires transfusion
	*BUN	<1.5 x N	1.5 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	>10 x N
	• **Urinary tract infection should be coded under infection, not GU.** • **Hematuria resulting from thrombocytopenia should be coded under hemorrhage, not GU.**					
GI	Nausea	none	able to eat reasonable intake	intake significantly decreased but can eat	no significant intake	- - - - -
	Vomiting	none	1 episode in 24 hours	2-5 episodes in 24 hours	6-10 episodes in 24 hours	>10 episodes in 24 hrs or requiring parenteral support
	Diarrhea	none	increase of 2-3 stools/day over pre-Rx	increase of 4-6 stools/day, or nocturnal stools, or moderate cramping	increase of 7-9 stools/day or incontinence, or severe cramping	increase of ≥10 stools/day or grossly bloody diarrhea, or need for parenteral support
	Stomatitis	none	painless ulcers, erythema, or mild soreness	painful erythema, edema, or ulcers, but can eat	painful erythema, edema or ulcers, and cannot eat	requires parenteral or enteral support
Liver	Bilirubin	WNL	- - - - -	<1.5 x N	1.5 - 3.0 x N	>3.0 x N
	Transaminase (SGOT, SGPT)	WNL	≤2.5 x N	2.6 - 5.0 x N	5.1 - 20.0 x N	>20.0 x N
	Alk Phos or 5'nucleotidase	WNL	≤2.5 x N	2.6 - 5.0 x N	5.1 - 20.0 x N	>20.0 x N
	Liver - clinical	no change from baseline	- - - - -	- - - - -	precoma	hepatic coma
	• **Viral Hepatitis should be coded as infection rather than liver toxicity.**					
Pulmonary	- - - - -	none or no change	asymptomatic, with abnormality in PFTs	dyspnea on significant exertion	dyspnea at normal level of activity	dyspnea at rest
	• **Pneumonia is considered infection and not graded as pulmonary toxicity unless felt to be resultant from pulmonary changes directly induced by treatment.**					
Cardiac	Cardiac dysrhythmias	none	asymptomatic, transient, requiring no therapy	recurrent or persistent, no therapy required	requires treatment	requires monitoring, or hypotension or ventricular tachycardia or fibrillation
	Cardiac funtion	none	asymptomatic, decline of resting ejection fraction by less than 20% of baseline value	asymptomatic, decline of resting ejection fraction by more than 20% of baseline value	mild CHF, responsive to therapy	severe or refractory CHF
	Cardiac-- ischemia	none	non-specific T-wave flattening	asymptomatic, ST and T wave changes suggesting ischemia	angina without evidence for infarction	acute myocardial infarction
	Cardiac-- pericardial	none	asymptomatic effusion, no intervention required	pericarditis (rub, chest pain, ECG changes)	symptomatic effusion; drainage required	tamponade; drainage urgently required

		0	1	2	3	4	
Blood Pressure		Hypertension	none or no change	asymptomatic, transient increase by >20 mm Hg (D) or to >150/100 if previously WNL. No treatment required	recurrent or persistent increase by >20 mm Hg (D) or to >150/100 if previously WNL. No treatment required	requires therapy	hypertensive crisis
		Hypotension	none or no change	changes requiring no therapy (including transient orthostatic hypotension)	requires fluid replacement or other therapy but not hospitalization	requires therapy and hospitalization; resolves within 48 hours of stopping the agent	requires therapy and hospitalization for >48 hours after stopping the agent
Skin		-----	none or no change	scattered macular or papular eruption or erythema that is asymptomatic	scattered macular or papular eruption or erythema with pruritus or other associated symptoms	generalized symptomatic macular, papular or vesicular eruption	exfoliative dermatitis or ulcerating dermatitis
Allergy		-----	none	transient rash, drug fever <38° C, 100.4° F	urticaria, drug fever ≥ 38°C, 100.4°F, mild bronchospasm	serum sickness, bronchospasm, requires parenteral meds	anaphylaxis
*Phlebitis			none	arm	thrombophlebitis, leg	hospitalization	embolus
Local			none	pain	pain and swelling, with inflammation or phlebitis	ulceration	plastic surgery indicated
Alopecia		-----	no loss	mild hair loss	pronounced or total hair loss	-----	-----
Weight gain/loss		-----	<5.0%	5.0 - 9.9%	10.0 - 19.9%	≥20%	-----
NEUROLOGIC	Sensory	neuro -- sensory	none or no change	mild paresthesias; loss of deep tendon reflexes	mild or moderate objective sensory loss; moderate paresthesias	severe objective sensory loss or paresthesias that interfere with function	-----
		neuro -- vision	none or no change	-----	-----	symptomatic subtotal loss of vision	blindness
		neuro -- hearing	none or no change	asymptomatic, hearing loss on audiometry only	tinnitus	hearing loss interfering with function but correctable with hearing aid	deafness, not correctable
	Motor	neuro -- motor	none or no change	subjective weakness; no objective findings	mild objective weakness without significant impairment of function	objective weakness with impairment of function	paralysis
		neuro -- constipation	none or no change	mild	moderate	severe	ileus >96 hours
	Psych	neuro -- mood	no change	mild anxiety or depression	moderate anxiety or depression	severe anxiety or depression	suicidal ideation
	Clinical	neuro -- cortical	none	mild somnolence or agitation	moderate somnolence or agitation	severe somnolence, agitation, confusion, disorientation or hallucinations	coma, seizures, toxic psychosis
		neuro -- cerebellar	none	slight incoordination, dysdiadokinesis	intention tremor, dysmetria, slurred speech, nystagmus	locomotor ataxia	cerebellar necrosis
		neuro -- headache	none	mild	moderate or severe but transient	unrelenting and severe	-----
Metabolic		Hyperglycemia	<116	116 - 160	161 - 250	251 - 500	>500 or ketoacidosis
		Hypoglycemia	>64	55 - 64	40 - 54	30 - 39	<30
		Amylase	WNL	<1.5 x N	1.5 - 2.0 x N	2.1 - 5.0 x N	>5.1 x N
		Hypercalcemia	<10.6	10.6 - 11.5	11.6 - 12.5	12.6 - 13.5	≥13.5
		Hypocalcemia	>8.4	8.4 - 7.8	7.7 - 7.0	6.9 - 6.1	≤6.0
		Hypomagnesemia	>1.4	1.4 - 1.2	1.1 - 0.9	0.8 - 0.6	≤0.5
Coagulation		Fibrinogen	WNL	0.99 - 0.75 x N	0.74 - 0.50 x N	0.49 - 0.25 x N	≤0.24 x N
		Prothrombin time	WNL	1.01 - 1.25 x N	1.26 - 1.50 x N	1.51 - 2.00 x N	>2.00 x N
		Partial thromboplastin time	WNL	1.01 - 1.66 x N	1.67 - 2.33 x N	2.34 - 3.00 x N	>3.00 x N

* denotes ECOG specific criteria